WO9808506

# Title:
# PHARMACEUTICAL COMPOUNDS


## Abstract:

The invention provides novel cryptophycin compounds which can be useful for disrupting the microtubulin system, as anti-neoplastic agents, antifungal, and for the treatment of cancer. The invention further provides a formulation for administering the novel cryptophycin compounds.

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(54) Title: PHARMACEUTICAL COMPOUNDS

(57) Abstract

The invention provides novel cryptophycin compounds which can be useful for disrupting the microtubulin system, as anti-neoplastic agents, antifungal, and for the treatment of cancer. The invention further provides a formulation for administering the novel cryptophycin compounds.

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# PHARMACEUTICAL COMPOUNDS

This invention relates to the fields of pharmaceutical and organic chemistry and provides novel cryptophycin compounds useful as anti-microtubule agents.

Neoplastic disease, characterized by the proliferation of cells not subject to the normal control of cell growth, are a major cause of death in humans and other mammals. Clinical experience in cancer chemotherapy has demonstrated that new and more effective drugs are desirable to treat these diseases. Such clinical experience has also demonstrated that drugs which disrupt the microtubule system of the cytoskeleton can be effective in inhibiting the proliferation of neoplastic cells.

The microtubule system of eucaryotic cells is a major component of the cytoskeleton and is a dynamic assembly and disassembly. Thus, heterodimers of tubulin are polymerized and form microtubule. Microtubules play a key role in the regulation of cell architecture, metabolism, and division. The dynamic state of microtubules is critical to their normal function. With respect to cell division, tubulin is polymerized into microtubules that form the mitotic spindle.

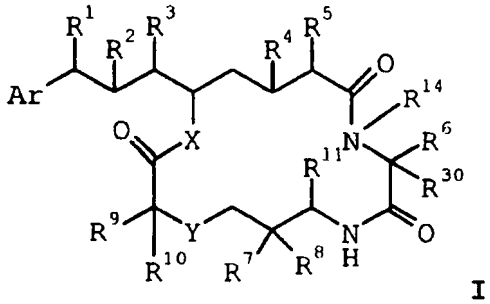
The microtubules are then depolymerized when the mitotic spindle's use has been fulfilled. Accordingly, agents which disrupt the polymerization or depolymerization of microtubules, and thereby inhibit mitosis, comprise some of the most effective cancer chemotherapeutic agents in clinical use.

Additionally, the compounds claimed herein possess fungicidal properties. Further, such agents having the

ability to disrupt the microtubule system can be useful for research purposes.

Certain cryptophycin compounds are known in the literature; however, cryptophycin compounds having even greater solubility, robust potency are desired for most pharmaceutical uses and a broader library of cryptophycin compounds could provide additional treatment options. Applicants have now discovered novel compounds providing such desired solubility as well as compounds having the ability to disrupt the microtubule system. Such compounds can be prepared using total synthetic methods and are, therefore, well suited for development as pharmaceutically useful agents.

The presently claimed invention provides novel compounds of Formula I



I

wherein

Ar is phenyl or any simple unsubstituted or substituted aromatic or heteroaromatic group, $C_1$-$C_{12}$ alkyl, $C_1$-$C_{12}$ alkyne;

$R^1$ is halogen, OH, $OR^{31}$, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylethio, dialkylsulfonium, sulfate, or phosphate;

$R^2$ is OH, $NH_2$, $NR^{31}$, SH; or

$R^1$ and $R^2$ may be taken together to form an epoxide ring, an aziridine ring, an episulfide ring, a sulfate ring, a cyclopropyl ring, or monoalkylphosphate ring; or

$R^1$ and $R^2$ may be taken together to form a second bond between $C_{18}$ and $C_{19}$;

$R^{31}$ is $C_1$-$C_6$ alkyl and hydrogen;

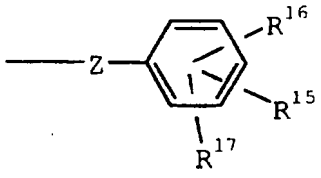
$R^3$ is a lower alkyl group;

$R^4$ is H;

$R^5$ is H;

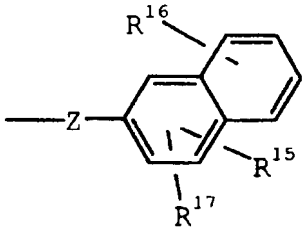
$R^4$ and $R^5$ may be taken together to form a second bond between $C_{13}$ and $C_{14}$;

$R^6$ is a substituent selected from the group consisting of B-ring heteroaromatic, substituted heteroaromatic, B-ring ($C_1$-$C_6$)alkyl, ($C_3$-$C_8$)cycloalkyl, substituted $C_3$-$C_6$ cycloalkyl, substituted ($C_1$-$C_6$)alkyl, a group of the formula **III'**:



**III'**

and a group of the formula **III''**:



**III'';**

$R^7$ is selected from the group consisting of $NR^{51}R^{52}$, $R^{53}NR^{51}R^{52}$, $OR^{53}$, H and a lower alkyl group; $R^{51}$ and $R^{52}$ are independently selected from the group consisting of $C_1$-$C_3$ alkyl; $R^{53}$ is $C_1$-$C_3$ alkyl;

$R^8$ is H or a lower alkyl group;

$R^7$ and $R^8$ can optionally form a cyclopropyl ring;

$R^9$ is selected from the group consisting of H, a lower alkyl group, unsaturated lower alkyl, and lower alkyl-$C_3$-$C_5$ cycloalkyl;

$R^{10}$ is H or a lower alkyl group;

$R^9$ and $R^{10}$ together optionally form a cyclopropyl ring;

$R^{11}$ is selected from the group consisting of H, OH, simple alkyl, phenyl, substituted phenyl, benzyl, and substituted benzyl;

$R^{14}$ is H or a lower alkyl group;

$R^{15}$, $R^{16}$ and $R^{17}$ are each independently selected from the group consisting of hydrogen, $(C_1-C_6)$alkyl, $OR^{18}$, halo, $NR^{18'}R^{19'}$, $NO_2$, $OPO_3H_2$, $OR^{19}$phenyl, $SCH_2$phenyl, $CONH_2$, $CO_2H$, $PO_3H_2$, and $SO_2R^{23}$, and ZZ;

$R^{18}$ is selected from the group consisting of hydrogen, aryl, and $C_1-C_6$ alkyl;

$R^{18'}$ is selected from the group consisting of hydrogen and $(C_1-C_6)$alkyl;

$R^{19}$ is $C_1-C_6$ alkyl;

$R^{19'}$ is selected from the group consisting of hydrogen and $(C_1-C_6)$alkyl

$R^{23}$ is selected from the group consisting of hydrogen and $(C_1-C_3)$alkyl;

$R^{29}$ is $(C_1-C_5)$alkyl;

$R^{30}$ is hydrogen or $C_1-C_6$ alkyl;

n is 0, 1, or 2;

p is 0, 1, or 2;

m is 0, 1, or 2;

X is selected from the group consisting of O, NH and alkylamino;

Y is selected from the group consisting of O, NH, and alkylamino;

Z is selected from the group consisting of $-(CH_2)_n-$, $-(CH_2)_p-O-(CH_2)_m-$ and $(C_3-C_5)$ cycloalkyl;

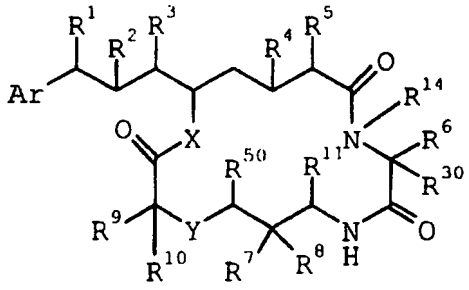
ZZ is selected from the group consisting of an aromatic group and a substituted aromatic group; or

a pharmaceutically acceptable salt or solvate thereof;

provided that when $R^6$ is a group of Formula III' and n is 1, then at least one of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ must be a non-hydrogen group and if only one of $R^{15}$, $R^{16}$ and

$R^{17}$ is OH or $OR^{29}$ and one of the group consisting of $R^{15}$, $r^{16}$ and $R^{17}$ is halo then the remaining member of the group consisting of $R^{15}$, $R^{16}$, and $R^{17}$ must not be hydrogen or halo; or when $R^6$ is a group of Formula III' and n is 1, $R^{14}$ is a lower alkyl group.

Further, the present invention provides compound of the formula I'



I'

wherein

Ar is phenyl or any simple unsubstituted or substituted aromatic or heteroaromatic group, $C_1$-$C_{12}$ alkyl, $C_1$-$C_{12}$ alkyne;

$R^1$ is halogen, OH, $OR^{31}$, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, or phosphate;

$R^2$ is OH, $NH_2$, $NR^{31}$, SH; or

$R^{31}$ is $C_1$-$C_6$ alkyl and hydrogen;

$R^1$ and $R^2$ may be taken together to form an epoxide ring, an aziridine ring, an episulfide ring, a sulfate ring, a cyclopropyl ring, or monoalkylphosphate ring; or

$R^1$ and $R^2$ may be taken together to form a second bond between $C_{18}$ and $C_{19}$;

$R^3$ is a lower alkyl group;

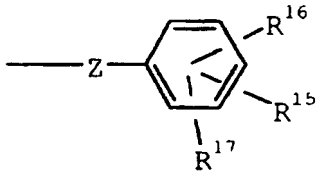
$R^4$ is H;

$R^5$ is H;

$R^4$ and $R^5$ may be taken together to form a second bond between $C_{13}$ and $C_{14}$;

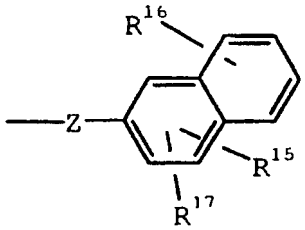
$R^6$ is a substituent selected from the group consisting of B-ring heteroaromatic, substituted heteroaromatic, B-ring ($C_1$-

$C_6$) alkyl, ($C_3$-$C_8$)cycloalkyl, substituted $C_3$-$C_8$ cycloalkyl, substituted ($C_1$-$C_6$) alkyl, a group of the formula **III'**:



**III'**

and a group of the formula **III''**:



**III''**;

$R^7$ is selected from the group consisting of H and a lower alkyl group;

$R^8$ is H or a lower alkyl group;

$R^7$ and $R^8$ can optionally form a cyclopropyl ring;

$R^9$ is selected from the group consisting of H, a lower alkyl group, unsaturated lower alkyl, and lower alkyl-$C_3$-$C_5$ cycloalkyl;

$R^{10}$ is H or a lower alkyl group;

$R^{50}$ is hydrogen or  ;

$R^{11}$ is selected from the group consisting of H, OH, simple alkyl, phenyl, substituted phenyl, benzyl, and substituted benzyl;

$R^{14}$ is H or a lower alkyl group;

$R^{15}$, $R^{16}$, and $R^{17}$ are each independently selected from the group consisting of hydrogen, ($C_1$-$C_6$)alkyl, $OR^{18}$, halo, $NR^{18'}R^{19'}$, $NO_2$, $OPO_3H_2$, $OR^{19}$phenyl, $SCH_2$phenyl, $CONH_2$, $CO_2H$, $PO_3H_2$, and $SO_2R^{23}$, and ZZ;

$R^{18}$ is selected from the group consisting of hydrogen, aryl, and $C_1$-$C_6$ alkyl;

$R^{18'}$ is selected from the group consisting of hydrogen and $(C_1-C_6)$alkyl;

$R^{19}$ is $C_1-C_6$ alkyl;

$R^{19'}$ is selected from the group consisting of hydrogen and $(C_1-C_6)$alkyl;

$R^{23}$ is selected from the group consisting of hydrogen and $(C_1-C_3)$ alkyl;

$R^{29}$ is $(C_1-C_5)$alkyl;

$R^{30}$ is hydrogen or $C_1-C_6$ alkyl;

R is hydrogen or a group of the formula  ;

n is 0, 1, or 2;

p is 0, 1, or 2;

m is 0, 1, or 2;

X is selected from the group consisting of O, NH and alkylamino;

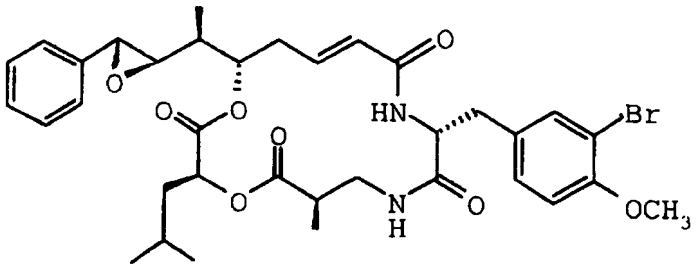
Y is selected from the group consisting of O, NH, and alkylamino;

Z is selected from the group consisting of $-(CH_2)_n-$, $-(CH_2)_p-O-(CH_2)_m-$ and $(C_3-C_5)$cycloalkyl;

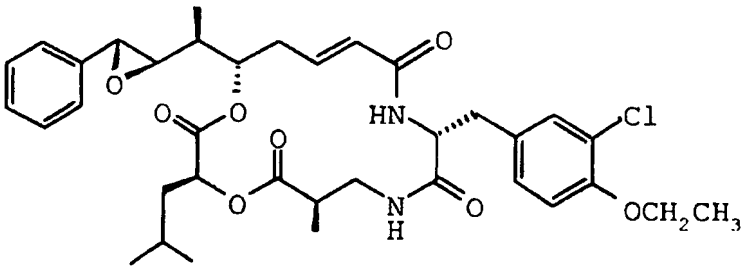
ZZ is selected from the group consisting of an aromatic group and a substituted aromatic group; or a pharmaceutically acceptable salt or solvate thereof;

provided that when $R^6$ is a group of Formula III' and n is 1, then at least one of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ must be a non-hydrogen group and if only one of $R^{15}$, $R^{16}$ and $R^{17}$ is OH or $OR^{29}$ and one of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ is halo then the remaining member of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ must not be hydrogen or halo; or when $R^6$ is a group of Formula III' and n is 1 then $R^{14}$ is lower alkyl;

further provided that the compound is not a cryptophycin selected from the group consisting of cryptophycins:


O
O
O
O
HN
Br
O
O
N
H
O
OCH3

B-2,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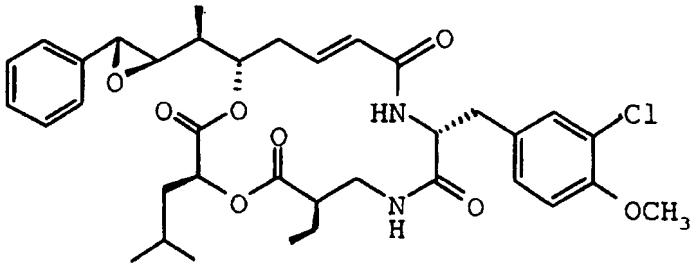
O
O
O
HN
Cl
O
O
N
H
O
OCH2CH3

B-7,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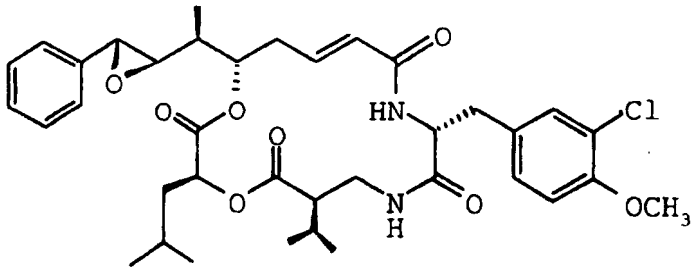
O
O
O
HN
Cl
O
O
N
H
O
OCH3

C-1,



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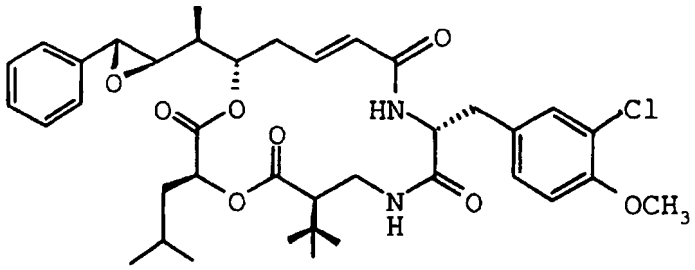

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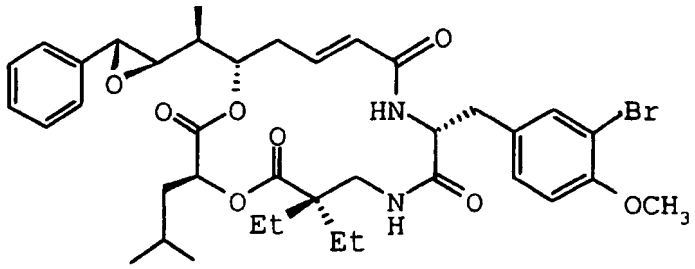

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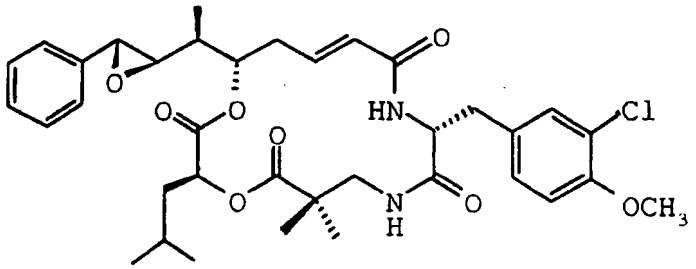


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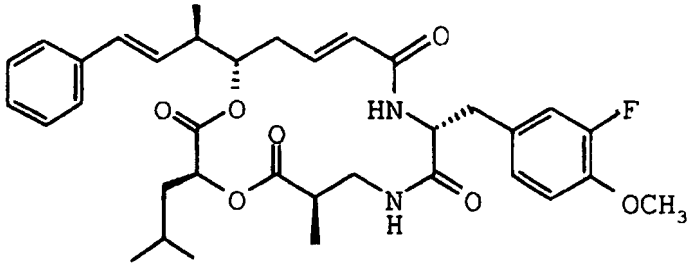


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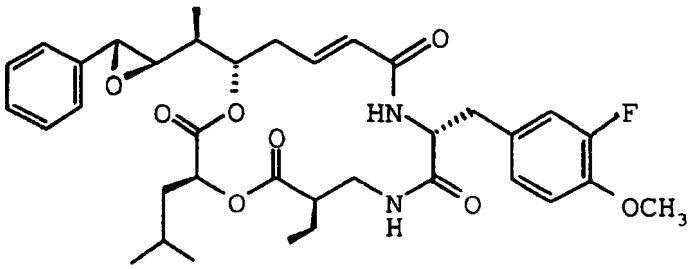


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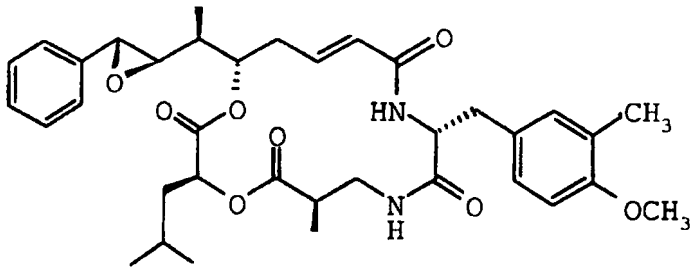

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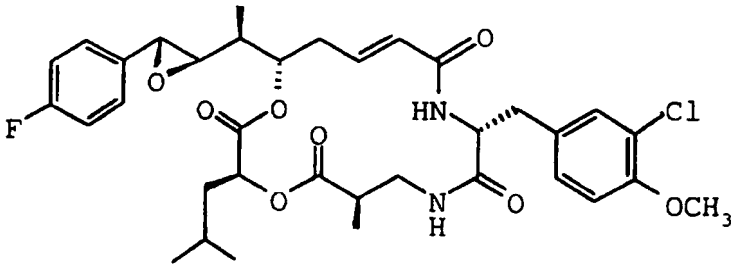

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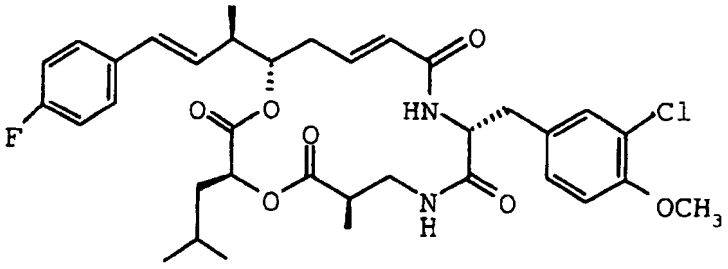

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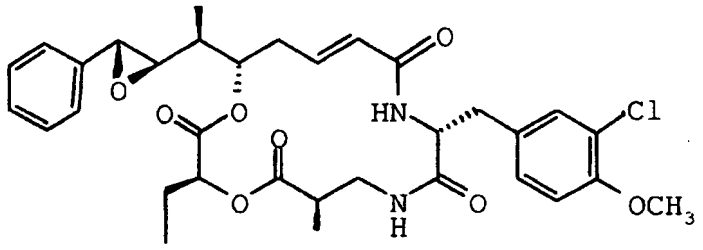

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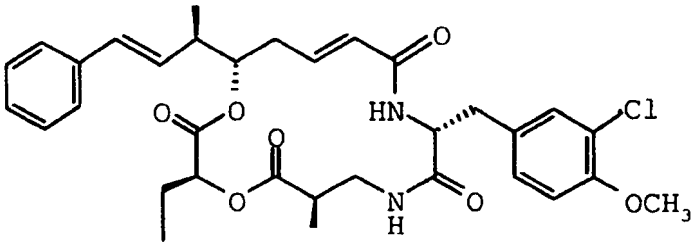

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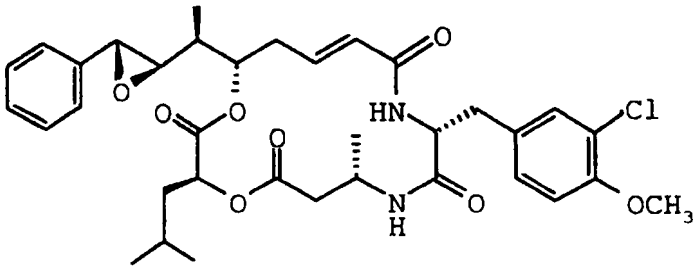

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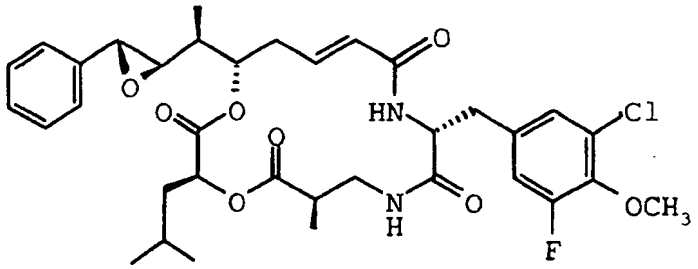

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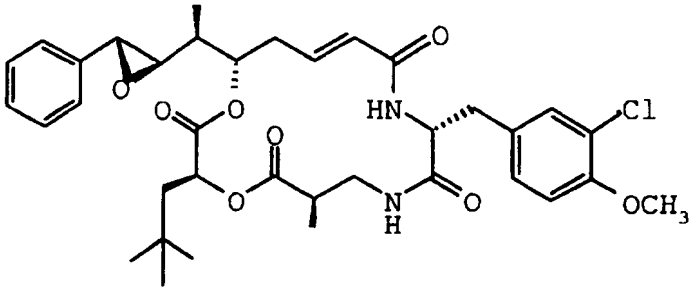


D-2

The present invention provides pharmaceutical formulations, a method for disrupting a microtubulin system using an effective amount of a compound of Formula I or I', a method for inhibiting the proliferation of mammalian cells comprising administering an effective amount of a compound of Formula I or I', and a method for treating neoplasia in a mammal comprising administering an effective amount of a compound of Formula I or I'.

As used herein, the term "simple alkyl" shall refer to $C_1$-$C_7$ alkyl wherein the alkyl may be saturated, unsaturated, branched, or straight chain. Examples include, but are in no way limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, propenyl, sec-butyl, n-pentyl, isobutyl,

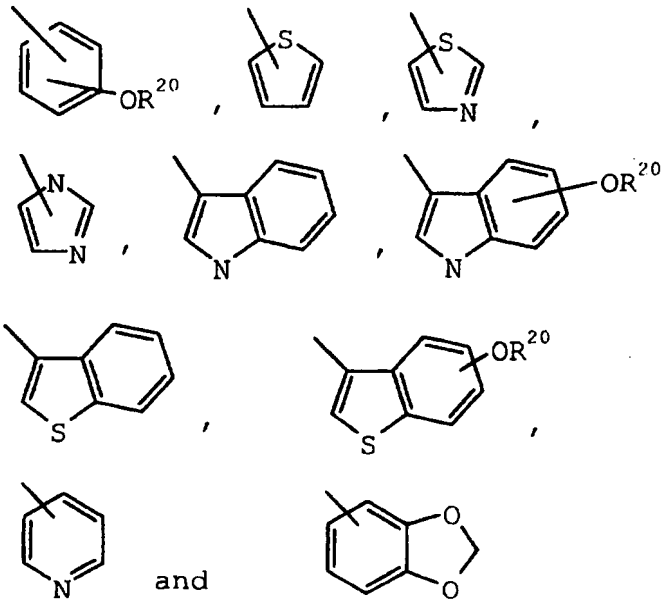
tert-butyl, sec-butyl, methylated butyl groups, pentyl, tert pentyl, sec-pentyl, methylated pentyl groups and the like.

As used herein, the term "B-ring $C_1$-$C_6$ alkyl" refers to saturated, unsaturated, branched and straight chain alkyl wherein the B-ring $C_1$-$C_6$alkyl group may include up to three (3) non-carbon substituents. Such non-carbon substituents are most preferably selected from the group consisting of OH, $SCH_2$phenyl, $NH_2$, CO, $CONH_2$, $CO_2H$, $PO_3H_2$, $SO_2R^{21}$ wherein $R^{21}$ is selected from hydrogen and $C_1$-$C_3$ alkyl;

As used herein, the term "substituted phenyl" shall refer to a phenyl group with from one to three non-hydrocarbon substituents which may be independently selected from the group consisting of simply alkyl, Cl, Br, F, and I.

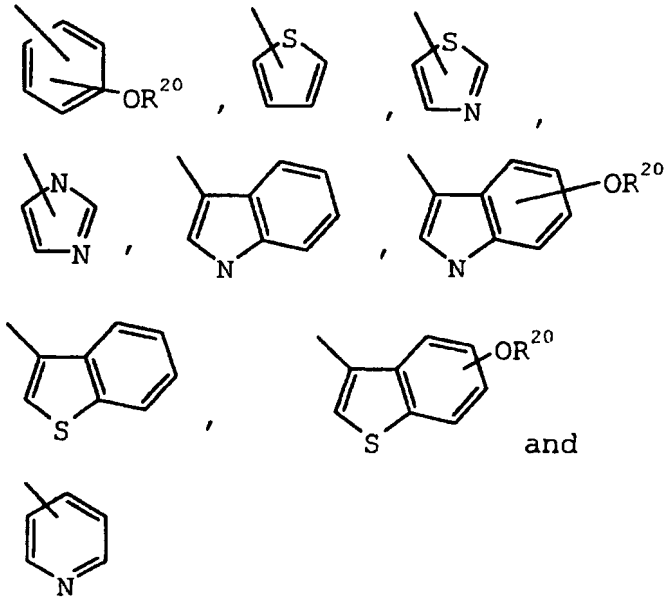
As used herein, the term "substituted benzyl" shall refer to a benzyl group with from one to three non-hydrocarbon substituents which may be independently selected from the group consisting of simply alkyl, Cl, Br, F, and I wherein such substituents may be attached at any available carbon atom.

As used herein "B-ring heteroaromatic group" refers to aromatic rings which contain one or more non-carbon substituent selected from the group consisting of oxygen, nitrogen, and sulfur. Especially preferred B-ring heterocyclic groups are selected from, but not limited to, the group consisting of:



wherein $R^{20}$ is selected from hydrogen and $C_1$-$C_6$ alkyl

It is especially preferred that "B-ring heteroaromatic group" refers to a substituent selected from the group consisting of:



As used herein, "cycloalkyl" refers to a saturated $C_1$-$C_8$ cycloalkyl group wherein such group may include from zero to three substituents selected from the group

consisting of $C_1$-$C_3$ alkyl, halo, and $OR^{22}$ wherein $R^{22}$ is selected from hydrogen and $C_1$-$C_3$ alkyl. Such substituents may be attached at any available carbon atom. It is especially preferred that cycloalkyl refers to substituted or unsubstituted cyclohexyl.

As used herein, "Lower alkoxyl group" means any alkyl group of one to five carbon atoms bonded to an oxygen atom. As used herein, "lower alkyl group" means an alkyl group of one to five carbons and includes linear and non-linear hydrocarbon chains, including for example, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, sec-butyl, methylated butyl groups, pentyl, tert pentyl, sec-pentyl, and methylated pentyl groups. As used herein, allylically substituted alkene" means any alkene having from one to seven carbon atoms which contain an alkyl substitution on it. As used herein, the term "unsaturated lower alkyl" means a lower alkyl group as defined *supra* wherein from one to two double bonds are present in the unsaturated lower alkyl substituent. A preferred unsaturated lower alkyl is $-CH_2-CH=CH_2$. The term "lower alkyl-$C_3$-$C_5$ cycloalkyl" refers to C-C alkyl substituted with a $C_3$-$C_5$cycloalkyl group. A preferred lower alkyl-$C_3$-$C_5$ cycloalkyl group is $-CH_2$-cyclopropyl; wherein the group is attached to the cryptophycin core structure at $R^9$ via the $CH_2$.

As used herein "epoxide ring" means a three-membered ring whose backbone consists of two carbons and an oxygen atom. As used herein, "aziridine ring" means a three-membered ring whose backbone consists of two carbon atoms and a nitrogen atom. As used herein, "sulfide ring" means a three-membered ring whose backbone consists of two carbon atoms and a sulfur atom. As used herein, "episulfide ring" means a three-membered ring whose backbone consists of two carbon atoms and a sulfur atom. As used herein,

"sulfate group" means a five-membered ring consisting of a carbon-carbon-oxygen-sulfur-oxygen backbone with two additional oxygen atoms connected to the sulfur atom. As used herein, "cyclopropyl ring" means a three-member ring whose backbone consists of three carbon atom. As used herein, "monoalkylphosphate ring" means a five-membered ring consisting of a carbon-carbon-oxygen-phosphorous-oxygen backbone with two additional oxygen atoms, one of which bears a lower alkyl group, connected to the phosphorous atom.

As used herein, "simple unsubstituted aromatic group" refers to common aromatic rings having 4n+2 electrons in a monocyclic conjugated system, for example, but not limited to: furyl, pyrrolyl, thienyl, pyridyl and the like, or a bicyclic conjugated system, for example, but not limited to: indolyl or naphthyl.

As used herein, "simple substituted aromatic group" refers to a phenyl group substituted with a single group selected from the group consisting of halogen and lower alkyl group.

As used herein, "heteroaromatic group" refers to aromatic rings which contain one or more non-carbon substituent selected from the group consisting of oxygen, nitrogen, and sulfur.

As used herein, "halogen" or "halo" refers to those members of the group on the periodic table historically known as halogens. Methods of halogenation include, but are not limited to, the addition of hydrogen halides, substitution at high temperature, photohalogenation, etc., and such methods are known to the skilled artisan.

As used herein, the term "mammal" shall refers to the Mammalia class of higher vertebrates. The term "mammal" includes, but is not limited to, a human. The term

"treating" as used herein includes phophylaxis of the named condition or amelioration or elimination of the condition once it has been established. The cryptophycin compounds claimed herein can be useful for veterinary health purposes as well as for the treatment of a human patient.

Some preferred characteristics of this invention are set forth in the following tabular form wherein the features may be independently selected to provide preferred embodiments of this invention. The invention is in no way limited to the features described below:

A) $R^8$ is ethyl, propyl, isopropyl, butyl, isobutyl or isopentyl;

B) $R^7$ is ethyl, propyl, isopropyl, butyl isobutyl, pentyl, or isopentyl;

C) $R^7$ is H, $R^8$ is methyl, $R^3$ is methyl, and X and Y are not both O;

D) $R^3$ is ethyl, propyl, isopropyl, butyl, isobutyl, pentyl or isopentyl;

E) $R^9$ is methyl, ethyl, propyl, butyl, isobutyl, pentyl, or isopentyl;

F) $R^{10}$ is methyl, ethyl, propyl, butyl, isobutyl, pentyl, or isopentyl;

G) a crytophycin compound wherein at least one of the groups selected from the group consisting of C-3, C-6, C-7, C-10, C-16, C-17, and C-18 has *R* stereochemistry (numbering as set forth in Formula I *supra*.);

H) a cryptophycin compound wherein at least one of the groups selected from the group consisting of C-3, C-6, C-7, C-10, C-16, C-17, and C-18 has *S* stereochemistry (numbering as set forth in Formula I *supra*.);

I) Ar is phenyl with a substituent selected from the group consisting of hydrogen, halogen, and simple alkyl;

J) a compound wherein Y is O'

K) a compound wherein Y is O, $R^7$, $R^8$, $R^9$ and $R^{10}$ are each hydrogen; and $R^1$ and $R^2$ form an epoxide;

L) $R^7$, $R^8$ are each hydrogen

M) $R^7$ and $R^8$ are each selected from hydrogen and $CH_3$;

5N) Y is O;

O) R is selected from the group consisting of methyl, ethyl, n-propyl, and phenyl;

P) $R^1$ and $R^2$ form an epoxide ring;

Q) both X and Y are O;

10R) $R^4$ and $R^5$ form a double bond;

S) n is 0; $R^6$ is substituted benzyl wherein one substituent is a halogen and one is an $OR^{12}$ group wherein $R^{12}$ is lower alkyl;

T) a compound of Formula I is used for disruption of a

15 microtubulin system;

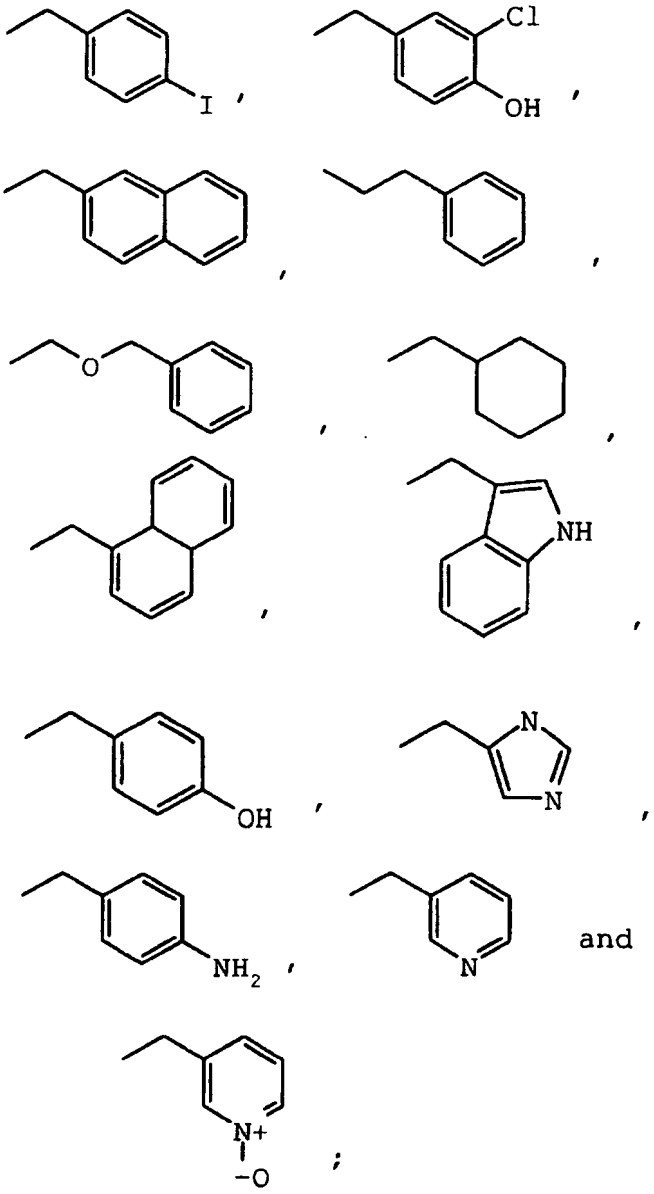
U) a compound of Formula I is used as an anti-neoplastic agent;

V) a compound of Formula I is used for the treatment of cancer in a mammal;

W) a compound of Formula I is used as an antifungal agent;

20X) $R^6$ is Formula **III'** and is para hydroxy substituted;

Y) $R^6$ is selected from the group consisting of



and

Z) Z is $-(CH_2)_n-$ wherein n is 0;

AA) Z is $-(CH_2)_n-$ wherein n is 2;

BB) Z is $-(CH_2)_n-$ wherein n is 1;

CC) $R^6$ is Formula III';

DD) $R^6$ is Formula III'';

EE) $R^6$ is $C_3$-$C_6$ cycloalkyl;

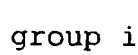
FF) $R^6$ is selected from the group consisting of B-ring heteroaromatic, substituted heteroaromatic, B-ring

alkyl, cycloalkyl, substituted cycloalkyl, Formula III' and Formula III'';

GG) at least one of $R^{15}$, $R^{16}$, and $R^{17}$ is selected from the group consisting of $SCH_2$phenyl, $NH_2$, CO, $CONH_2$, $CO_2H$, $PO_3H_2$, and $SO_2R^{21}$; wherein $R^{21}$ is selected from hydrogen and $C_1$-$C_3$ alkyl;

HH) Ar is phenyl;

II) Ar is phenyl substituted with one or two from the group consisting of OH, $OCH_3$, halo, and methyl; and Ar is naphthyl;

KK) $R^6$ has a Z wherein the first carbon of the Z group is  with respect to the point of attachment to the cryptophycin molecule;

LL) $R^6$ is a heteroaromatic ring;

MM) $R^7$ is selected from the group consisting of $N(CH_3)_2$, $CH_2N(CH_3)_2$;

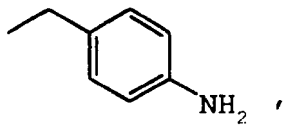
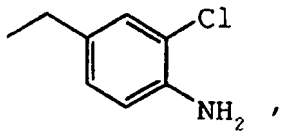
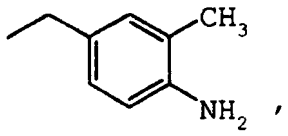
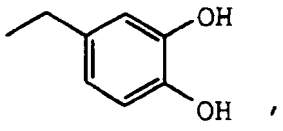
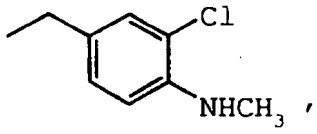
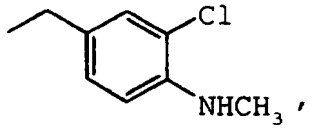
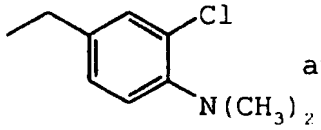
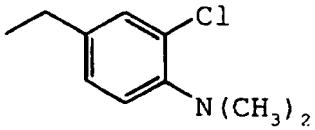
NN) $R^7$ is $CH_2OCH_3$;

OO) $R^7$ is cyclopropyl;

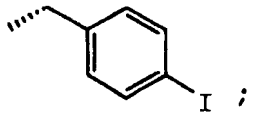
PP) $R^9$ is $CH_2$cyclopropyl;

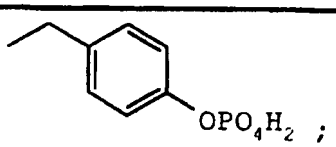
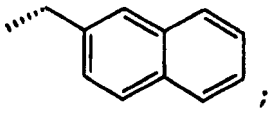
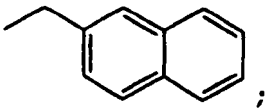
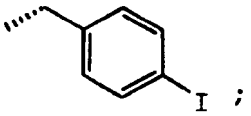
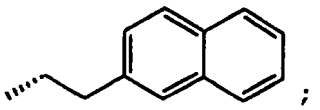
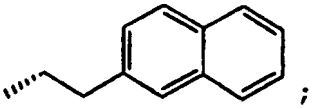
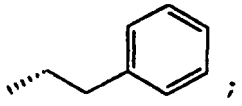
QQ) $R^9$ is $CH_2CH=CH_2$;

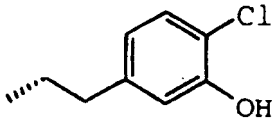
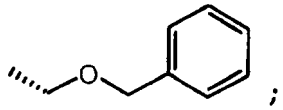
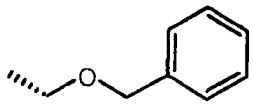
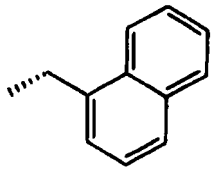
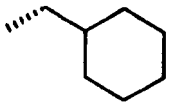
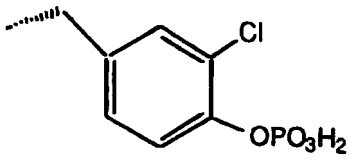
RR) $R^6$ is selected from the group consisting of

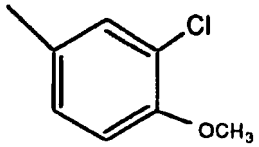







and


To further illustrate, but to no way limit, the compounds contemplated herein, the following table of especially preferred compounds is provided: A compound wherein $R^3$ is $CH_3$; $R^4$ and $R^5$ together form a second bond; $R^{14}$ is hydrogen; $R^{30}$ is hydrogen; $R^7$ and $R^8$ are each methyl; $R^{10}$ is hydrogen; $R^{10}$ is hydrogen; $R^9$ is $-CH_2CH(CH_3)_2$; X and Y are each O; Ar is phenyl; and

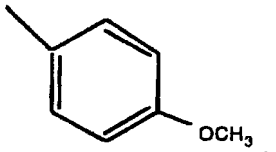
| $R^1$ | $R^2$ | $R^6$ |
|---|---|---|
| together form a double bond | |  ; |

| $R^1$ | $R^2$ | $R^6$ |
|---|---|---|
| | |  ; |
| together form an epoxide | | |
| | |  ; |
| together form an epoxide | | |
| | |  ; |
| together form a double bond | | |
| | |  ; |
| Cl | OH | |
| | |  ; |
| Cl | OH | |
| | |  ; |
| together form a double bond | | |
| | |  ; |
| together form an epoxide | | |

| $R^1$ | $R^2$ | $R^6$ |
|---|---|---|
| | |  ; |
| Cl | OH | |
| | |  ; |
| together form a double bond | | |
| | |  ; |
| Cl | OH | |
| | |  ; |
| together form a double bond | | |
| | |  ; |
| together form a double bond | | |
| | |  . |
| Cl | OH | |

Additional preferred compounds are those named above except that Ar is  instead of phenyl.

Further preferred compounds are those named above except that

Ar is  .

The present invention provides a method of alleviating a pathological condition caused by hyperproliferating mammalian cells comprising administering to a subject an effective amount of a pharmaceutical or veterinary composition disclosed herein to inhibit proliferation of the cells. In a preferred embodiment of this invention, the method further comprises administering to the subject at least one additional therapy directed to alleviating the pathological condition. In a preferred embodiment of the present invention, the pathological condition is characterized by the formation of neoplasms. In a further preferred embodiment of the present invention, the neoplasms are selected from the group consisting of mammary, small-cell lung, non-small-cell lung, colorectal, leukemia, melanoma, pancreatic adenocarcinoma, central nervous system (CNS), ovarian, prostate, sarcoma of soft tissue or bone, head and neck, gastric which includes pancreatic and esophageal, stomach, myeloma, bladder, renal, neuroendocrine which includes thyroid and non-Hodgkin's disease and Hodgkin's disease neoplasms.

As used herein "neoplastic" refers to a neoplasm, which is an abnormal growth, such growth occurring because of a proliferation of cells not subject to the usual limitations of growth. As used herein, "anti-neoplastic agent" is any compound, composition, admixture, co-mixture, or blend which inhibits, eliminates, retards, or reverses the neoplastic phenotype of a cell.

Anti-mitotic agents may be classified into three groups on the basis of their molecular mechanism of action. The first group consists of agents, including colchicine and colcemid, which inhibit the formation of microtubules by sequestering tubulin. The second group consists of agents,

including vinblastine and vincristine, which induce the formation of paracrystalline aggregates of tubulin. Vinblastine and vincristine are well known anticancer drugs: their action of disrupting mitotic spindle microtubules preferentially inhibits hyperproliferative cells. The third group consists of agents, including taxol, which promote the polymerization of tubulin and thus stabilizes microtubules.

The exhibition of drug resistance and multiple-drug resistance phenotype by many tumor cells and the clinically proven mode of action of anti-microtubule agents against neoplastic cells necessitates the development of anti-microtubule agents cytotoxic to non-drug resistant neoplastic cells as well as cytotoxic to neoplastic cells with a drug resistant phenotype.

Chemotherapy, surgery, radiation therpy, therapy with biological response modifiers, and immunotherapy are currently used in the treatment of cancer. Each mode of therapy has specific indications which are known to those of ordinary skill in the art, and one or all may be employed in an attempt to achieve total destruction of neoplastic cells. Moreover, combination chemotherapy, chemotherapy utilizing compounds of Formula I in combination with other neoplastic agents, is also provided by the subject invention as combination therapy is generally more effective than the use of a single anti-neoplastic agent. Thus, a further aspect of the present invention provides compositions containing a therapeutically effective amount of at least one compound of Formula I, including the non-toxic addition salts thereof, which serve to provide the above recited benefits. Such compositions can also be provided together with physiologically tolerable liquid, gel, or solid carriers, diluents, adjuvants and excipients. Such carriers, adjuvants, and excipients may be found in the U.S. *Pharmacopeia*, Vol. **XXII** and National Formulary vol **XVII**, U.S. *Pharmacopeia* Convention, Inc. Rockville, MD (1989). Additional modes of treatment are provided in AHFS Drug

Information, 1993 e. by the American Hospital Formulary Service, pp. 522-660. Each of these references are well known and readily available to the skilled artisan.

The present invention further provides a pharmaceutical composition used to treat neoplastic disease containing at least one compound of Formula I and at least one additional anti-neoplastic agent. Anti-neoplastic agents which may be utilized in combination with Formula I compounds include those provided in the Merck Index **11**, pp 16-17, Merck & Co., Inc. (1989). The Merck Index is widely recognized and readily available to the skilled artisan.

In a further embodiment of this invention, antineoplastic agents may be antimetabolites which may include but are in no way limited to those selected from the group consisting of methotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine, arabinoside, hydroxyurea, and 2-chlorodeoxyadenosine. In another embodiment of the present invention, the anti-neoplastic agents contemplated are alkylating agents which may include but are in no way limited to those selected from the group consisting of cyclophosphamide, mephalan, busulfan, paraplatin, chlorambucil, and nitrogen mustard. In a further embodiment, the anti-neoplastic agents are plant alkaloids which may include but are in no way limited to those selected from the group consisting of vincristine, vinblastine, taxol, and etoposide. In a further embodiment, the anti-neoplastic agents contemplated are antibiotics which may include, but are in no way limited to those selected from the group consisting of doxorubicin, daunorubicin, mitomycin C, and bleomycin. In a further embodiment, the anti-neoplastic agents contemplated are hormones which may include, but are in no way limited to those selected from the group consisting of calusterone, diomostavolone, propionate, epitiostanol, mepitiostane, testolactone, tamoxifen, polyestradiol phosphate, megesterol acetate, flutamide, nilutamide, and trilotane.

In a further embodiment, the anti-neoplastic agents contemplated include enzymes which may include, but are in no way limited to those selected from the group consisting of L-Asparginase and aminoacridine derivatives such as, but not limited to, amsacrine. Additional anti-neoplastic agents include those provided by Skeel, Roland T., "Antineoplastic Drugs and Biologic Response Modifier: Classification, Use and Toxicity of Clinically Useful Agents" Handbook of Cancer Chemotherapy (3rd ed.), Little Brown & Co. (1991).

These compounds and compositions can be administered to mammals for veterinary use. For example, domestic animals can be treated in much the same way as a human clinical patient. In general, the dosage required for therapeutic effect will vary according to the type of use, mode of administration, as well as the particularized requirements of the individual hosts. Typically, dosages will range from about 0.001 to 1000 mg/kg, and more usually 0.01 to 10 mg/kg of the host body weight. Alternatively, dosages within these ranges can be administered by constant infusion over an extended period of time, usually exceeding 24 hours, until the desired therapeutic benefits are obtained. Indeed, drug dosage, as well as route of administration, must be selected on the basis of relative effectiveness, relative toxicity, growth characteristics of tumor and effect of Formula I compound on cell cycle, drug pharmacokinetics, age, sex, physical condition of the patient and prior treatment, which can be determined by the skilled artisan.

The compound of Formula I, with or without additional anti-neoplastic agents, may be formulated into therapeutic compositions as natural or salt forms. Pharmaceutically acceptable non-toxic salts include base addition salts which may be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as

isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. Such salts may also be formed as acid addition salts with any free cationic groups and will generally be formed with inorganic acids such as for example, hydrochloric or phosphoric acids or organic acids such as acetic, oxalic, tartaric, mandelic, and the like. Additional excipients which further the invention are provided to the skilled artisan for example in the U.S. *Pharmacopeia*.

The suitability of particular carriers for inclusion in a given therapeutic composition depends on the preferred route of administration. For example, anti-neoplastic compositions may be formulated for oral administration. Such compositions are typically prepared as liquid solution or suspensions or in solid forms. Oral formulation usually include such additives as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers, mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained relsease formulations, or powders, and typically contain 1% to 95% of active ingedient. More preferably, the composition contains from about 2% to about 70% active ingredient.

Compositions of the present invention may be prepared as injectables, either as liquid solutions, suspensions, or emulsions; solid forms suitable for solution in or suspension in liquid prior to injection. Such injectables may be administered subcutaneously, intravenously, intraperitoneally, intramuscularly, intrathecally, or intrapleurally. The active ingredient or ingredients are often mixed with diluents, carriers, or excipients which are physiologically tolerable and compatible with the active ingredient(s). Suitable diluents and excipients are for example, water, saline, dextrose,

glycerol, or the like and combinations thereof. In addition, if desired, the compositions may contain minor amounts of auxilary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents.

The invention further provides methods for using Formula I compounds to inhibit the proliferation of mammalian cells by contacting these cells with a Formula I compound in an amount sufficient to inhibit the proliferation of the mammalian cell. A preferred embodiment is a method to inhibit the proliferation of hyperproliferative mammalian cells. For purposes of this invention "hyperproliferative mammalian cells" are mammalian cells which are not subject to the characteristic limitations of growth (programmed cell death for example). A further preferred embodiment is when the mammalian cell is human. The invention further provides contacting the mammalian cell with at least one Formula I compound and at least one anti-neoplastic agent. The types of anti-neoplastic agents contemplated are discussed *supra*.

The invention further provides methods for using a compound of Formula I to inhibit the proliferation of hyperproliferative cells with drug-resistant phenotypes, including those with multiple drug-resistant phenotypes, by contacting said cell with a compound of Formula I in an amount sufficient to inhibit the proliferation of a hyperproliferative mammalian cell. A preferred embodiment is when the mammalian cell is human. The invention further provides contacting a Formula I compound and at least one additional anti-neoplastic agent, discussed *supra*.

The invention provides a method for alleviating pathological conditions caused by hyperproliferating mammalian cells for example, neoplasia, by administering to a subject an effective amount of a pharmaceutical composition containing Formula I compound to inhibit the proliferation of the hyperproliferating cells. As used herein "pathological condition" refers to any pathology

arising from the proliferation of mammalian cells that are not subject to the normal limitations of growth. Such proliferation of cells may be due to neoplasms as discussed *supra*.

In a further preferred embodiment the neoplastic cells are human. The present invention provides methods of alleviating such pathological conditions utilizing a compound of Formula I in combination with other therapies, as well as other anti-neoplastic agents.

The effectiveness of the claimed compounds can be assessed using standard methods known to the skilled artisan.

Examples of such methods are as follows:

Compounds of this invention have been found to be useful against pathogenic fungi. For example, the usefulness for treating *Cryptococcus neoformans* can be illustrated with test results against *Cryptococcus neoformans* employing yeast nitrogen base detrose agar medium. In carrying out the assay, a compound of this invention is solubilized in dimethyl sulfoxide supplemented with Tween 20. Twofold dilutions are made with sterile distilled water/10 percent DMSO to obtain final drug concentrations in the agar dilution assay plates ranging from 0.008 µg/ml to 16.0 µg/ml against an expanded panel of 84 *Cryptococcus neoformans* strains. The minimum inhibitory concentration against the panel of 84 *Cryptococcus neoformans* isolates is determined to illustrate the desired antifungal activity.

The compounds are screened for minimum inhibitory concentrations against KB, a human nasopharyngeal carcinoma cell line, LoVo, a human colorectal adenocarcinoma cell line using The Corbett assay, see Corbett, T.H. et al. Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development, pp 35-87, Kluwer Academic Publishers: Norwell, 1992. see also, Valeriote, et al. Discovery and Development

of Anticancer Agents; Kluwer Academic Publishers, Norwell, 1993 is used for the evaluation of compounds.

The most active compounds are further evaluated for cytotoxicity against four different cell types, for example a murine leukemia, a murine solid tumor, a human solid tumor, and a low malignancy fibroblast using the Corbett assay.

The compounds are further evaluated against a broad spectrum of murine and human tumors implanted in mice, including drug resistant tumors.

Tumor burden (T/C) (mean tumor burden in treated animals versus mean tumor burden in untreated animals) are used as a further assessment. T/C values that are less than 42% are considered to be active by National Cancer Institute Standards; T/C values less than 10% are considered to have excellent activity and potential clinical activity by National Cancer Institute standards.

Materials

Vinblastine, cytochalasin B, tetramethylrhodamine isothiocyanate (TRITC)-phalloidin, sulforhodamine B (SRB) and antibodies against ß-tubulin and vimentin are commercially available from recognized commercial vendors. Basal Medium Eagle containing Earle's salts (BME) and Fetal Bovine Serum (FBS) are also commercially available.

Cell Lines

The Jurkat T cell leukemia line and A-10 rat aortic smooth muscle cells are obtained from the American Type Culture Collection and are cultured in BME containing 10% FBS and 50µg/mL gentamycin sulfate. Human ovarian carcinoma cells (SKOV3) and a sub-line which has been selected fro resistance to vinblastine (SKVLB1) were a generous gift from Dr. Victor Ling of the Ontario Cancer Institute. Both cell lines are maintained in BME containing 10% FBS and 50µg/mL gentamycin sulfate. Vinblastine is added to a final

concentration of 1µg/mL to SKVLB1 cells 24 hours after passage to maintain selection pressure for P-glycoprotein-overexpressing cells.

Cell Proliferation and Cycle Arrest Assays

Cell proliferation assays are performed as described by Skehan *et al.* For Jurkat cells, cultures are treated with the indicated drugs as described in Skehan and total cell numbers are determined by counting the cells in a hemacytometer. The percentage of cells in mitosis are determined by staining with 0.4% Giemsa in PBS followed by rapid washes with PBS. At least 1000 cells per treatment are scored for the presence of mitotic figures and the mitotic index is calculated as the ration of the cells with mitotic figures to the total number of cells counted.

Immunofluorescence Assays

A-10 cells are grown to near-confluency on glass coverslips in BME/10% FBS. Compounds in PBS are added to the indicated final concentrations and cells are incubated for an additional 24 hours. For the staining of microtubules and intermediate filaments, the cells are fixed with cold methanol and incubated with PBS containing 10% calf serum to block nonspecific binding sites. Cells are then incubated at 37_C for 60 min. with either monoclonal anti-ß-tubulin or with monoclonal anti-vimentin at dilutions recommended by the manufacturer. Bound primary antibodies are subsequently visualized by a 45-minute incubation with fluorescein-conjugated rabbit antimouse IgG. The coverslips are mounted on microscope slides and the fluorescence patterns are examined and photographed using a Zeiss Photomicroscope Ill equipped with epifluorescence optics for fluorescein. For staining of microfilaments, cells are fixed with 3% paraformaldehyde, permeabilized with 0.2% Triton X-100 and chemically reduced with sodium borohydride (1mg/ML). PBS containing 100nM TRITC-phalloidin is then

added and the mixture is allowed to incubate for 45 min. at 37_C. The cells are washed rapidly with PBS before the coverslips are mounted and immediately photographed as described above.

Effects of cryptophycins and vinblastine on Jurkat cell proliferation and cell cycle

Dose-response curves for the effects of cryptophycin compounds and vinblastine on cell proliferation and the percentage of cells in mitosis are determined.

Effects of cytochalasin B, vinblastine and cryptophycins on the cytoskeleton

Aortic smooth muscle (A-10) cells are grown on glass coverslips and treated with PBS, 2μM cytochalasin B, 100nM vinblastine or 10nM cryptophycin compounds . After 24 hours, microtubules and vimentin intermediate filaments are visualized by indirect immunofluorescence and microfilaments are stained using TRITC - phalloidin. The morphological effects of each drug is examined. Untreated cells displayed extensive microtubule networks complete with perinuclear microtubule organizing centers. Vimentin intermediate filaments were also evenly distributed throughout the cytoplasm, while bundles of microfilaments were concentrated along the major axis of the cell. Cytochalasin B caused complete depolymerization of microfilaments along with the accumulation of paracrystalline remnants. This compound did not affect the distribution of either microtubules or intermediate filaments. The cryptophycin treated microtubules and vimentin intermediates are observed for depletion of microtubules, and collapse of rimentin intermediate filaments.

Effects of cryptophycins and vinblastine on taxol-stabilized microtubules

A-10 cells are treated for 3 hours with 0 or 10μM taxol before the addition of PBS, 100nM vinblastine or 10nM cryptophycin compound. After 24 hours, microtubule organization is examined by immunofluorescence as described above. Compared with those in control cells, microtubules in taxol-treated cells were extensively bundled, especially in the cell polar regions. As before, vinblastine caused complete depolymerization of microtubules non-pretreated cells. However, pretreatment with taxol prevented microtubule depolymerization in response to vinblastine. Similarly, microtubules pretreated with taxol are observed with cryptophycin treatment.

Reversibility of microtubule depolymerization by vinblastine and cryptophycin

A-10 cells are treated with either 100nM vinblastine or 10nM cryptophycins for 24 hr., resulting in complete microtubule depolymerization. The cells are then washed and incubated in drug-free medium for periods of 1 hour or 24 hours. Microtubules repolymerized rapidly after the removal of vinblastine, showing significant levels of microtubules after 1 hour and complete morphological recovery by 24 hour. Cells are visualized for microtubule state after treatment with a cryptophycin compound of this invention at either 1 hour or 24 hours after removal of the cryptophycin compounds.

Effects of combinations of vinblastine and cryptophycins on cell proliferation

SKOV3 cells are treated with combinations of cryptophycins and vinblastine for 48 hours. The percentages of surviving cells are then determined and the $IC_{50}$s for each combination is calculated.

Toxicity of cryptophycins, vinblastine and taxol toward SKOV3 and SKVLB1 cells

SKVLB1 cells are resistant to natural product anticancer drugs because of their over expression of P-glycoprotein. The abilities of taxol, vinblastine and cryptophycin compounds to inhibit the growth of SKOV3 and SKVLB1 cells are observed. Taxol caused dose-dependent inhibition of the proliferation of both cell lines with $IC_{50}$s for SKOV3 and SKVLB1 cells of 1 and 8000nM, respectively. Vinblastine also inhibited the growth of both cell lines, with $IC_{50}$s of 0.35 and 4200nM for SKOV3 and SKVLB1 cells, respectively. Cryptophycins compounds of this invention demonstrate activity with an $IC_{50}$s of from about 1 to about 1000pm for SKOV3 and SKVLB1 cells.

Thus it can be demonstrated that the present invention provides novel cryptophycin compounds which are potent inhibitors of cell proliferation, acting by disruption of the microtubule network and inhibition of mitosis. These studies can illustrate that cryptophycin compounds disrupt microtubule organization and thus normal cellular functions, including those of mitosis.

Classic anti-microtubule agents, such as colchicine and *Vinca* alkaloids, arrest cell division at mitosis. It seems appropriate to compare the effect of one of these agents on cell proliferation with the cryptophycin compounds. For this purpose, the *Vinca* alkaloid vinblastine was selected as representative of the classic anti-microtubule agents. Accordingly, the effect of cryptophycin compounds and vinblastine on the proliferation and cell cycle progression of the Jurkat T-cell leukemia cell line is compared.

Since antimitotic effects are commonly mediated by disruption of microtubules in the mitotic spindles, the effects of cryptophycin compounds on cytoskeletal structures are characterized by fluorescence microscopy. Immunofluorescence staining of cells treated with either a cryptophycin compound or vinblastine demonstrate that both

compounds cause the complete loss of microtubules. Similar studies with SKOV3 cells can show that the anti-microtubule Effects of cryptophycin compounds are not unique to the smooth muscle cell line.

## GC3 Human Colon Carcinoma Screen

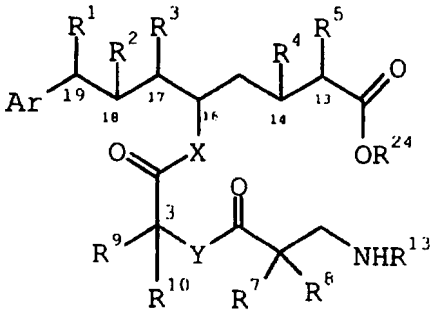
Selected wells of a 96 well plate were seeded with GC3 human colon carcinoma cells (1x10 cells in a 100µl assay medium/well) twenty-four hours prior to test compound addition. Cell free assay medium was added to other select wells of the 96 well plate. The assay medium (RPMI-1640 was the medium used; however, any medium that will allow the cells to survive would be acceptable) was supplemented with 10% dialyzed fetal bovine serum and 25 MM HEPES buffer.

The test compound was stored in an amber bottle prior to testing. Fresh dimethylsulfoxide stock solution (200µg/ml) was prepared immediately prior to preparation of test sample dilutions in phosphate-buffered saline (PBS). A dilution of 1:20 dimethylsulfoxide solution in PBS was prepared such that the final concentration was 10 µg/ml. Serial 1:3 dilutions using PBS (.5ml previous sample of 1ml PBS) were prepared. Falcon 2054 tubes were used for the assay.

A 10µl sample of each dilution of test compound was added in triplicate to wells of GC3 plates. The plates were incubated for 72 hours at about 37°C. A 10µl sample of stock 3-[4,5-dimethyl-2-yl]-2,5-diphenyltetrazolium bromide salt ("MTT" 5mg/ml in PBS) was added to each well. The plates were incubated for about an hour at 37°C. The plates were centrifuged, media was decanted from the wells and 100µl acid-isopropanol (0.04 N HCl in isopropanol) was added to each well. The plate was read within one hour using a test wavelength of 570nm (SpectraMax reader).

Evaluation of compounds of Formula I suggest that the compounds can be useful in the treatment methods claimed herein. Further, the compounds will be useful for disrupting the microtubule system.

Compounds of Formula I can be prepared using a compound of the Formula II



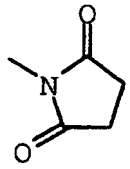
II

wherein

Ar, $R^1$, $R^2$, $R^3$, $R^4$, $R^5$, $R^6$, $R^7$, $R^8$, $R^9$, $R^{10}$ have the meanings set for *supra* in Formula I.

$R^{13}$ is selected from the group consisting of t-butylcarbamate (BOC);

$R^{24}$ is selected from the group consisting of

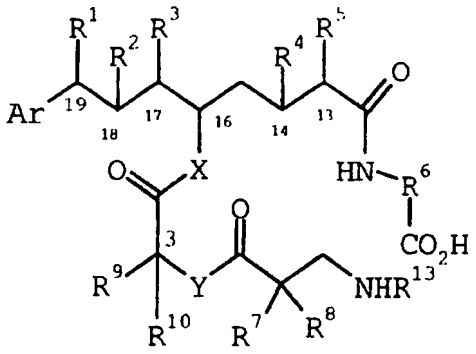


(N-hydroxysuccinimide, herein "NHS"), N-hydroxysulfosuccinimide and salts thereof, 2-nitrophenyl, 4-nitrophenyl, and 2,4-dichlorophenyl;

X is O, NH or alkylamino;

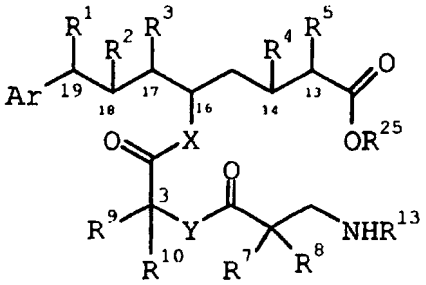
Y is O, NH, or alkylamino.

Compounds of Formula **III**



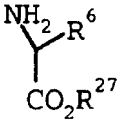
III

wherein the R groups and various substituents are as defined hereinbefore and throughout the specification; can be prepared by contacting a compound of the Formula **IV**



IV

$R^{25}$ is an active ester substituent;
with an acid of the formula



;

$R^{27}$ is selected from the group consisting of H, $C_1$-$C_{12}$ alkyl, and aryl;

and a silylating agent. Bis N, O-trimethylsilyl acetamide (BSA) is an especially preferred silylating agent.

As used with regard to $R^{25}$ the phrase "active ester substituent" refers to a substituent which makes the $OR^{24}$ substituent a good leaving group. Appropriate substituents can be selected with guidance from standard reference guides, for example, "Protective Groups in Organic Chemistry", Plenum Press, (London and New York, 1973); Greene, T.W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). An especially preferred $R^{25}$ group is N-hydroxy-succinimide. (NHS)

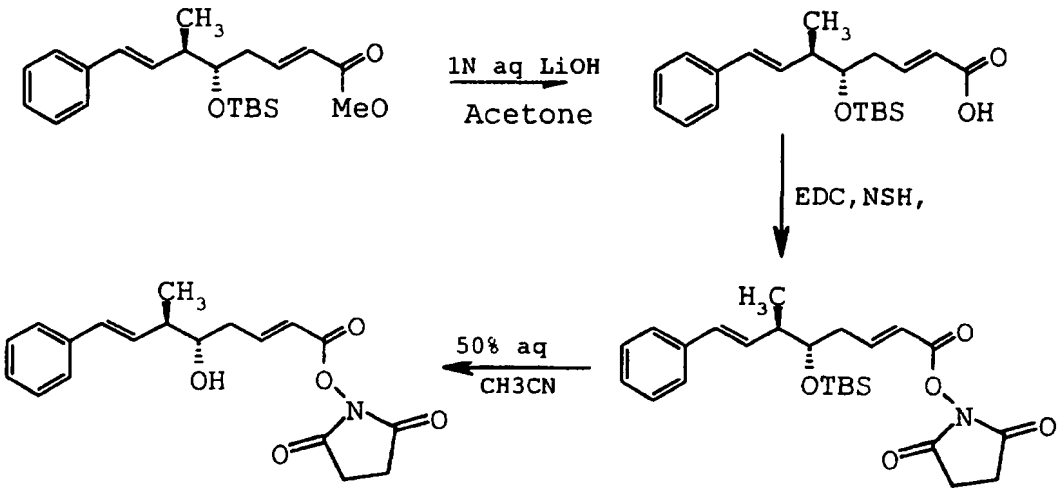
The processes described herein are most preferably completed in the presence of a solvent. The artisan can select an appropriate solvent for the above described process. Inert organic solvents are particularly preferred; however, under certain conditions an aqueous solvent can be appropriate. For example, if $R^{27}$ is hydrogen and $R^{13}$ is BOC an aqueous base as solvent will be effective.

When the desired $R^6$ substituent in the compound of Formula I contains an amine, then the amine substituent of the $R^6$ group must be protected using an amino protecting group. The artisan can readily select an appropriate amino protecting group using guidance from standard works, including for example, "protective Groups in Organic Chemistry", Plenum Press, (London and New York, 1973); Greene, T.W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981).

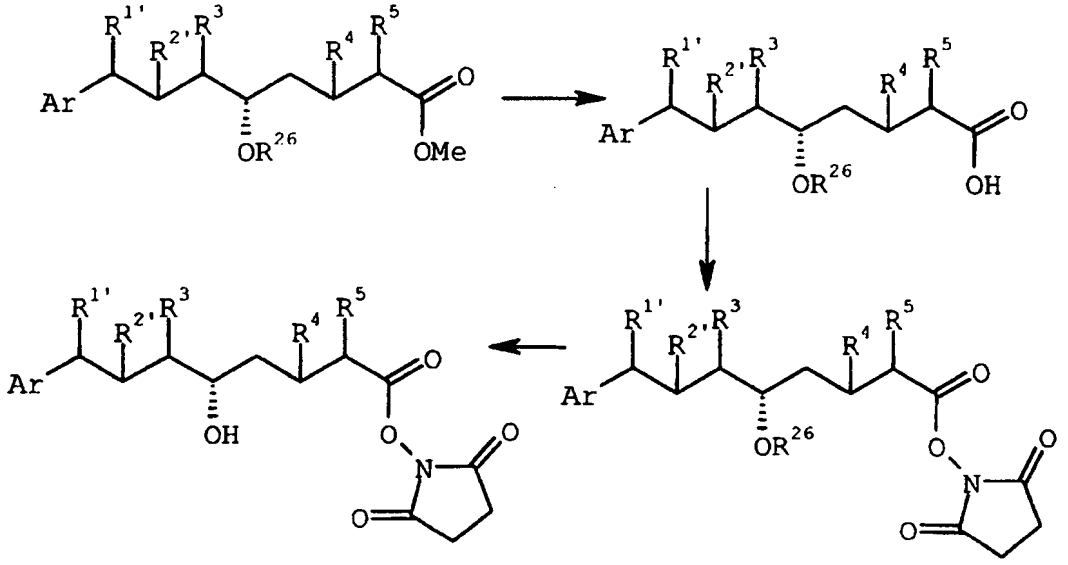
$R^{27}$ should be a group that allows for the removal of the $-CO_2R^{27}$ substituent using acidic, neutral, or mild basic conditions. Preferred $R^{27}$ groups include, but are in no way limited to, hydrogen, $C_1$-$C_6$ alkyl, trichloromethyl, trichloroethyl, and methylthiomethyl. It is especially preferred that $R^{27}$ is hydrogen.

To provide further guidance for the artisan, the following schemes are provided:

**Scheme 1**

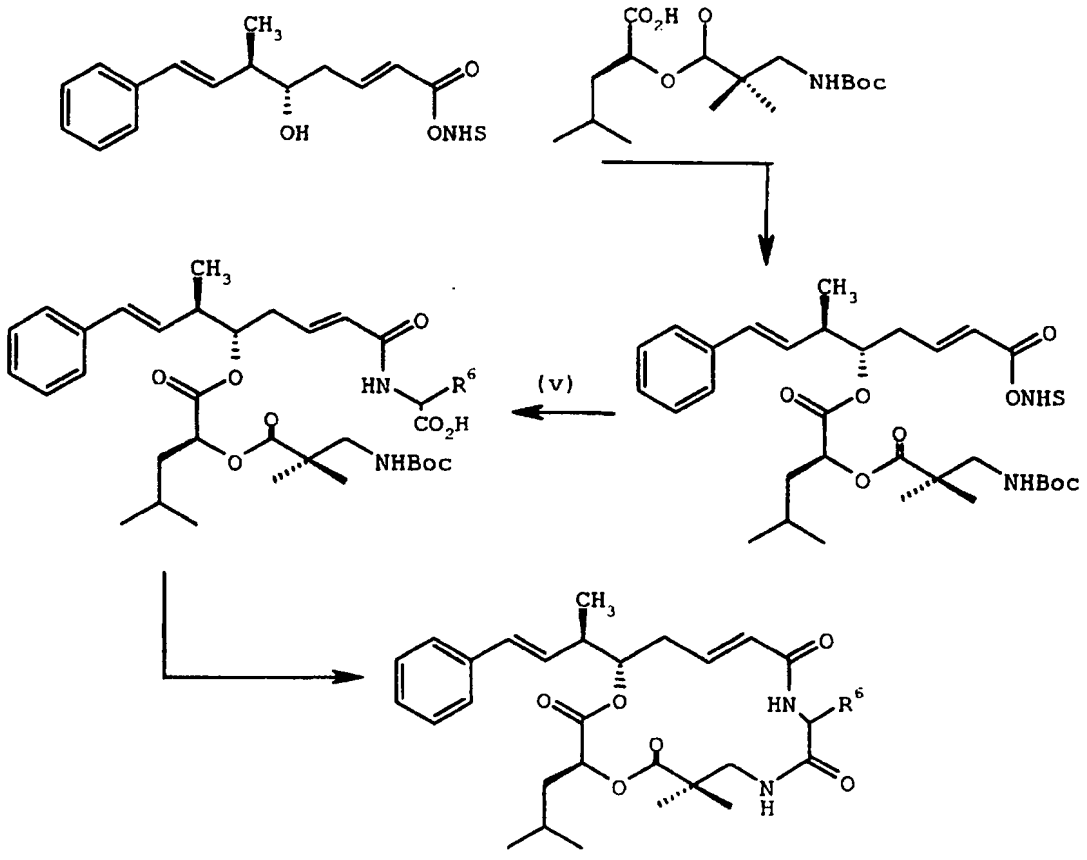



**Scheme I'**

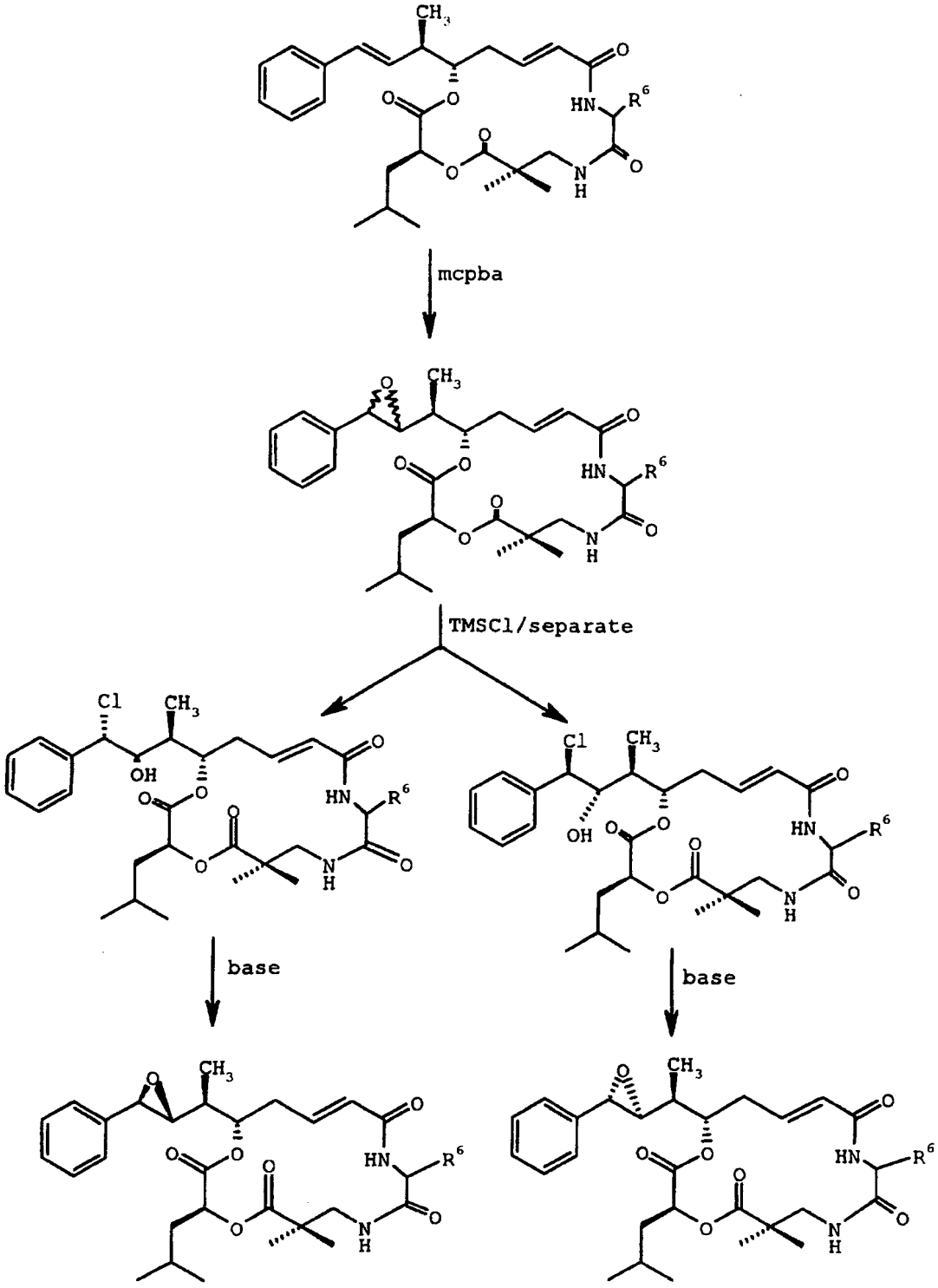



As used in Scheme I' and throughout the specification, $R^{1'}$ is halogen, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, phosphate or a protected OH or protected SH group;

$R^2$ is OH or SH; $R^{26}$ is an alcohol protecting group introduced during a portion of the synthetic process to protect an alcohol group which might otherwise react in the course of chemical manipulations, and is then removed at a later state of the synthesis. Numerous reactions for the formation and removal of such protecting groups are described in a number of standard works, including, for example, "protective Groups in Organic Chemistry", Plenum Press, (London and New York, 1973); Greene, T.W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). The skilled artisan can select an appropriate alcohol protecting group particularly with guidance provided from such works. One particularly useful alcohol protecting group is tert-butyldimethylsilyl (TBS).



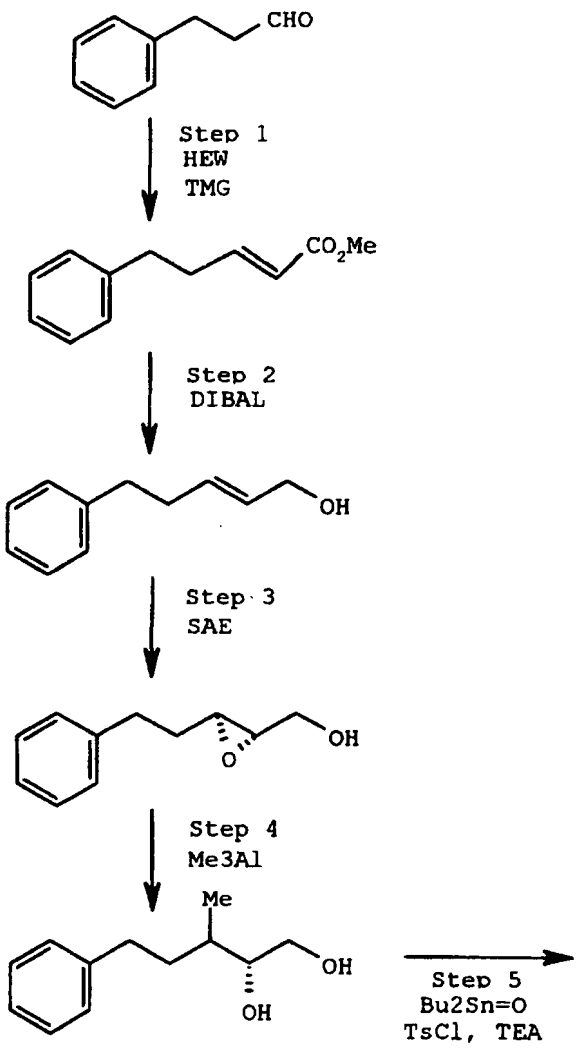

$R^6$ has the meaning defined *supra*.

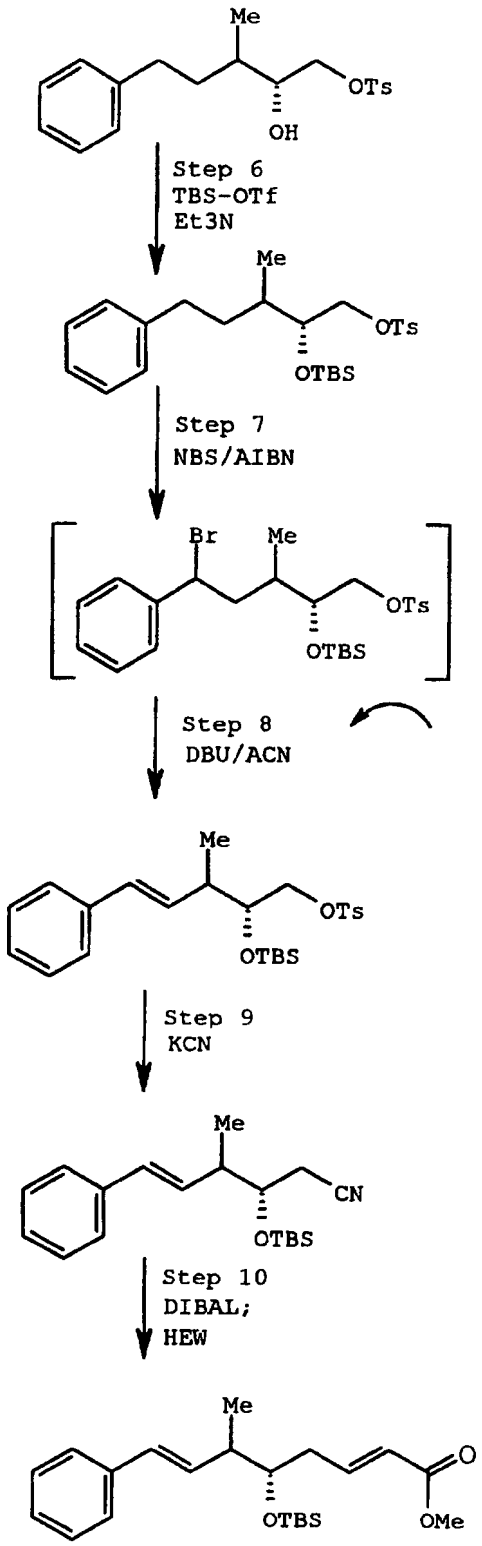

mcpba
TMSCl/separate
base
base

The product of the schemes provided herein can be further derivatized using standard methods to provide further cryptophycin compounds.

The artisan can utilize appropriate starting materials and reagents to prepare desired compounds using the guidance of the previous schemes and following examples.

The ester starting material can be prepared, for example, as follows:






$R^6$ has the meaning defined *supra*.

The scheme for preparing the ester is further explained by the Preparation Section herein which provides one specific application of the scheme for the convenience of the skilled artisan.

The Scheme for preparing the ester is applicable to the Ar substituents claimed herein. The scheme illustration is not intended to limit the synthesis scheme only to the phenyl ring illustrated. Rather, the artisan can broadly apply this process to provide desired starting materials for the compounds claimed herein.

The necessary reaction time is related to the starting materials and operating temperature. The optimum reaction time for a given process is, as always, a compromise which is determined by considering the competing goals of throughput, which is favored by short reaction times, and maximum yield, which is favored by long reaction times.

To further illustrate the invention the following examples are provided The scope of the invention is in no way to be construed as limited to or by the following examples.

## Preparation 1

**Step 1. Methyl 5-Phenylpent-2(*E*)-enoate.** A solution of trimethyl phosphonoacetate (376 g, 417 mL, 2.7 mol) in THF (750 mL) was stirred at 0°C in a 3L 3-neck round bottom flask equipped with a mechanical stirrer and $N_2$ inlet. To the chilled solution, neat tetramethyl guanidine (239 g, 260 mL, 2.07 mol) was added dropwise via an addition funnel. The chilled clear pale yellow solution was stirred for 25 minutes at 0°C. A solution of hydrocinnamaldehyde (90%, 253 g, 248 mL, 1.9 mol) in THF (125 mL) was added dropwise to the reaction solution slowly. Upon completion of addition, the reaction was stirred for 10 h rising to room

temperature. GC indicated at 95:5 ratio of product to starting material. 500 ml of water was added to the reaction vessel and the reaction stirred overnight separating into two layers. The organic layer was isolated and the aqueous layer was extracted with t-BuOMe. The organic layers were combined and dried over $MgSO_4$, then concentrated *in vacuo* to yield an orange oil. The crude product was distilled at 129°C/0.3mm Hg yielding 360.5g, 91.7% yield, of a clear slightly yellow oil.
EIMS *m/z* 190(13; M+), 159(410, 158(39), 131(90), 130(62), 117(22), 104(12), 95(57), 91(100), 77(21), 65(59); HREIMS *m/z* 190.0998 ($C_{12}H_{14}O_2$ D -0.4 mnu); UV lmax (e) 210 (8400), 260 (230) nm; IR nmax 3027, 2949, 1723, 1658, 1454, 1319, 1203, 978, 700 $cm^{-1}$; $^1H$ NMR d ($CDCl_3$) 7.15-7.3 (Ph-H5;bm), 7.00 (3-H;dt, 15.6/6.6), 5.84 (2-H;dt, 15.6/1.2), 3.70 (OMe;s), 2.76 (5-H2;t, 7.2), 2.51 (4-H2; bdt, 6.6/7.2); $^{13}C$ NMR d ($CDCl_3$) 166.9 (1), 148.3(3), 140.6(Ph-1'), 128.4/128.2 (Ph2'/3'/5'6'), 126.1 (Ph 4'), 121.4 (2). 51.3 (OMe), 34.2/33.8 (4/5).

**Step 2. 5-phenyl-pent-2-en-1-ol.** To a 12L 4-neck round bottom flask equipped with a thermocouple, mechanical stirrer and $N_2$ inlet, a solution of enoate ester (310.5 g, 1.5 mol) in THF (1.5 L) was charged and chilled to -71°C via a *i*-PrOH/$CO_2$ bath. To the reaction vessel, was added dropwise DIBAL (2.5 L, 1.5 M in toluene, 3.75 mol) at a rate to maintain the reaction temperature < -50°C. Upon complete addition, the reaction was stirred overnight with the reaction temperature < -50°C. TLC (3:1 Hexanes:EtOAc, $SiO_2$) indicated absence of starting material after 16 h. The reaction temperature was allowed to raise to -15°C. The reaction was quenched slowly with1N HCl (150 mL). At this point the reaction setup into a gelatinous solid. A spatula was employed to breakup the the semi-solid and 1N HCl (200 mL) was added making the mixture more fluid. Concentrated HCl (625 mL) was charged to form a two phase system. The

layers were separated and the product extracted with t-BuOMe. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield a clear pale yellow oil, 247.8g. The crude product was distilled at 145°C/0.25mm Hg yielding 209.7g, 86.2%.
EIMS *m/z* 162 (1:M+) 144 (16), 129 (7), 117 (9) 108 (6), 92 (17), 91 (100), 75 (5), 65 (12), HREIMS *m/z* 162, 1049 ($C_{11}H_{14}O$, D -0.4 mmu); UV lmax (e) 206 (9900), 260 (360); IR nmax 3356, 2924, 1603, 1496, 1454, 970, 746, 700 $cm^{-1}$; $^1H$ NMR d 7.15-7.3 (Ph-H5;m), 5.70 (3-H;dt, 15.6/6.0), 5.61 (2-H;dt, 15.6/4.8), 4.02 (1-H2;d 4.8), 2.68 (5-H2; t, 7.2), 2.40 (OH;bs), 2.36(4-H2; dt, 6.0/7.2); $^{13}C$ NMR d141.6 (Ph 1'), 131.8(3), 129.5 (2), 128.3/128.2 (Ph 2'/3'/5'/6'), 125.7 (Ph 4'), 63.3 (1), 35.4/33.8 (4/5).

**Step 3. (2S,3S)-2,3-Epoxy-5-phenyl-1-pentanol.** To a 1L 3 neck round bottom flask equipped with a mechanical stirrer, thermocouple and nitrogen inlet was added $CH_2Cl_2$ (350 mL), dried 4 Å molecular sieves (30 g) and L-(+)-diethyl tartrate (7.62 g, 0.037 mol). The resulting mixture was cooled to -20 °C and treated with $Ti(O\text{-}i\text{-}Pr)_4$ (9.2 mL, 0.031 mol), followed by the addition of *t*-butylhydroperoxide (4.0 M in $CH_2Cl_2$, 182 mL, 0.78 mol) at a rate to maintain the temperature ≤ -20°C. Upon complete addition, the reaction mixture was stirred for another 30 min, and then treated with a solution of the allylic alcohol (50 g, 0.31 mol) in $CH_2Cl_2$ (30 mL) at a rate to maintain the temperature ≤ -20°C. The reaction was stirred at the same temperature for 5 h, then filtered into a solution of ferrous sulfate heptahydrate (132 g) and tartaric acid (40 g) in water (400 mL) at 0 °C. The mixture was stirred for 20 min, then transferred to a separatory funnel and extracted with *t*-BuOMe (2x200 mL). The combined organic phase was stirred with 30% NaOH solution containing NaCl, for 1 h at 0°C. The layers were again separated, and the aqueous phase extracted with *t*-BuOMe. The combined organic phase was washed with

brine, dried over $MgSO_4$ and concentrated to yield 52.8 g as an amber oil.

**Step 4. (2*R*, 3*R*)-2-hydroxy-3-methyl-5-phenylpentan-1-ol .** To a 5L 3 neck round bottom flask equipped with a mechanical stirrer, thermocouple and nitrogen inlet was added hexanes (1L) and cooled to 0°C. A 2.0M solution of $Me_3Al$ in hexanes (800 mL, 1.6 mol) was added, followed by a solution of the epoxide (120 g, 0.677 mol) in hexanes (250 mL)/$CH_2Cl_2$ (50 mL) maintaining the temperature below 20°C. Upon complete addition, the cloudy reaction mixture was stirred at 5°C for 35 min, whereupon a solution of 10% HCl (300 mL) was added dropwise, followed by the addition of concd HCl (350 mL). The layers were separated, and the organic phase was washed with brine and dried over $MgSO_4$. After removal of the volatiles *in vacuo*, 122.1 gram of an oil was obtained.

**Step 5. (2*R*, 3*R*)-2-hydroxy-3-methyl-5-phenylpent-1-yl Tosylate.** To a 2L 3 neck round bottom flask equipped with a mechanical stirrer and nitrogen inlet was added the diol (58 g, 0.30 mol), dibutyltin oxide (1.5 g, 0.006 mol, 2 mol%), toluenesulfonyl chloride (57.5 g, 0.30 mol), $CH_2Cl_2$ (580 mL) and triethylamine (42.0 mL, 0.30 mol). The resulting mixture was stirred at room temperature for 2 h (although the reaction was complete within 1 h), filtered, washed with water and dried over $MgSO_4$. Concentration of the volatiles *in vacuo* afforded 104.1 gram of a slightly amber oil.

**Step 6. (2*R*, 3*R*)-2-[(*tert*-Butyldimethylsilyl)oxy]-3-methyl-5-phenylpent-1-yl Tosylate.** A solution of the tosylate (100 g, 0.29 mol) and triethylamine (81.0 mL, 0.58 mol) in $CH_2Cl_2$ (1200 mL) was treated with neat TBS-OTf (99 mL, 0.43 mol) dropwise with continued stirring for another 20 min. The reaction was washed twice with brine, dried over $MgSO_4$ and concentrated to dryness. The oil was dissolved in a minimal amount of hexanes and filtered over a silica pad, eluting

with hexanes:EtOAc (9:1) to yield a slightly amber oil, 134 g.

**Step 7. (2*R*, 3*R*,5*RS*)-2-[(*tert*-Butyldimethylsilyl)oxy]-3-methyl-5-bromo-5-phenylpent-1-yl Tosylate.** To a 5L 3 neck round bottom flask equipped with a mechanical stirrer, reflux condenser and nitrogen inlet was added $CCl_4$ (1680 mL), TBS Ts (140 g, 0.30 mol), NBS (65g, 0.365 mol) and AIBN (16.5 g, 0.10 mol). The mixture was degassed by evacuation under full vacuum with stirring, and backfilling with nitrogen (3x). The reaction mixture was then heated to reflux, whereupon the color became dark brown. After 15 min at vigorous reflux, the reaction mixture became light yellow, and chromatographic analysis indicated the reaction was complete. After cooling to room temperature, the reaction was filtered and the filtrate concentrated to dryness. The residue was redissolved in hexanes and filtered again, and concentrated to dryness to afford 170.3 gram as an amber oil.

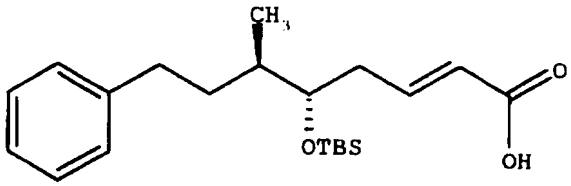
**Step 8. (2*R*, 3*R*)-2-[(*tert*-Butyldimethylsilyl)oxy]-3-methyl-5-phenylpent-4(*E*)-en-1-yl Tosylate.** To a 2L 3 neck round bottom flask equipped with a mechanical stirrer, reflux condenser and nitrogen inlet was added a solution of the bromide (100 g, 0.186 mol) in acetonitrile (700 mL). DBU (83.6 mL, 0.557 mol) was added and the resulting dark brown solution was stirred at reflux for 15 min. After cooling to room temperature, the solvent was removed *in vacuo*, and the residue digested in $CH_2Cl_2$ (200 mL) and filtered through a silica pad. The volatiles were again evaporated, and the residue dissolved in EtOAc and washed with water, brine and dried over $MgSO_4$ and concentrated to dryness. Preparative mplc (Prep 500) chromatography afforded the desired unsaturated compound (50.3 g, 60% yield over 4 steps).

**Step 9. (3*S*, 4*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-4-methyl-6-phenylhex-5(*E*)-en-1-nitrile.** The tosylate (50 g, 0.11 mol) was dissolved in DMSO (1 L) and treated with KCN (14.2 g, 0.22 mol) and water (25 mL), and the resulting mixture was stirred at 60°C under nitrogen for 18 h. After cooling to room temperature, the reaction mixture was partitioned between EtOAc (1 L) and water (1 L). The aqueous phase was extracted with EtOAc (500 mL), and the combined organic phase was washed with brine and dried over $Na_2SO_4$. Flash chromatography over silica with $CH_2Cl_2$ afforded the desired nitrile in 92% yield.

**Step 10. Methyl (5*S*, 6*R*)-5-[(*tert*-Butyldimethylsilyl)oxy]-6-methyl-8-phenylocta-2(*E*),7(*E*)-dienoate.** The nitrile (14.67 g, 46.5 mmol) was dissolved in toluene (200 mL) and cooled to -78°C under nitrogen. A 1.5M solution of DIBAL in toluene (37.2 mL, 55.8 mmol) was added dropwise with vigorous stirring. Upon complete addition, the cooling bath was removed and the reaction was stirred at room temperature for 1 h. The reaction mixture was carefully poured into 1N HCl and the mixture stirred at room temperature for 30 min. The layers were separated, and the organic phase was washed with a saturated aqueous solution of sodium potassium tartrate (2x), brine and dried over $Na_2SO_4$. The volatiles were removed *in vacuo*, and the crude pale yellow oil was used directly in the subsequent condensation. The crude aldehyde from above was dissolved in THF (90 mL) and treated with trimethyl phosphonoacetate (9.03 mL, 55.8 mmol) and tetramethylguanidine (7.0 mL, 55.8 mmol) at room temperature under nitrogen. The reaction mixture was stirred for 16 h, then partitioned between EtOAc (200 mL) and water (100 mL). The aqueous phase was back extracted with EtOAc (100 mL), and the combined organic phase was washed with water, brine and dried over $Na_2SO_4$. The volatiles were removed *in vacuo*, and the crude yellow oil (17.0 g) was chromatographed over

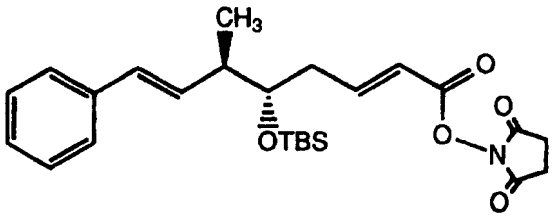
silica gel with $CH_2Cl_2$ : cyclohexane (1 : 1 to 2 : 1) to afford 13.67 grams of the desired ester, 78.5%.

**Preparation 2**




Methyl ester (2.673 mmol) was dissolved in acetone and then 1N aqueous LiOH (26mL) added at room temperature. The cloudy mixture was further diluted with acetone (20mL) and the resulting yellow mixture stirred at room temperature for 23.5h. The reaction was diluted with diethylether (400mL) and the organics washed with 1N HCl (120mL), brine (200mL) and $H_2O$ (160mL). The organics were dried and concentrated *in vacuo* to leave a yellow oil which was purified by column chromatography (gradient: 5% AcOH + 20%-40% EtOAc/Hexanes ) to give carboxylic acid as a yellow oil (960mg, 100%).
$^1H$ NMR ($CDCl_3$) d 7.38-7.19 (m,$PhH_5$), 7.09 (ddd,J=15.2,7.6 and7.9 Hz,3-H), 6.38 (d,J=16 Hz,8-H), 6.16 (dd,J=16 and8 Hz, 7-H), 5.85 (d,J=15.8Hz,2-H),3.81-3.75 (m,5-H), 2.49-2.37 (m,6-H,4-$CH_2$), 1.12 (d,J=6.7Hz,6-Me), 0.91 (s,$SiCMe_3$), 0.065 (s,SiMe), 0.068 (s,SiMe) ppm;
IR u ($CHCl_3$) 2957,2930,2858,1697,1258,1098,838 $cm^{-1}$;
MS (FD) 360.2 ($M^+$,100);
[a]$_D$+87.6° (c 10.5, $CHCl_3$);
Anal. calcd. for $C_{21}H_{32}O_3$ requires: C,69.95; H,8.95%. Found: C,69.19; H,8.39%.

**Preparation 3**




To a stirred solution of carboxylic acid (2mmol) in dry dimethylformamide (5.50mL) was added 1-ethyl-3-(3-dimethyaminopropyl)carbodiimide (2.4mmol) and N-hydroxysuccinimide (2.6mmol) at room temperature. The mixture was stirred for 28h and then diluted with EtOAc (100mL) and washed with 1N aqueous HCl (2x50mL), $H_2O$ (75mL), dried and concentrated *in vacuo* to leave an oil. Crude product was purified by column chromatography (gradient: 5-30% EtOAc/Hexanes) to give active ester as a pale yellow oil (724mg,80%).

$^1H$ NMR ($CDCl_3$) d 7.36-7.20 (m,$PhH_5$,3-H), 6.38 (d,J=16Hz,8-H), 6.14 (dd,J=16.1 and 8.0 Hz,7-H). 6.03 (d,J=16Hz,2-H), 3.79 (q,J=4.3Hz,5-H), 2.94 (brs,$CH_2CH_2$), 2.58-2.42 (m,6-H,4-$CH_2$), 1.10 (d,J=6.8Hz,6-Me), 0.90 (s,$SiCMe_3$), 0.05 (s,$SiMe_2$) ppm;

IR u ($CHCl_3$)

2957,2931,2858,1772,1741,1648,1364,1254,1092,1069,838 $cm^{-1}$;

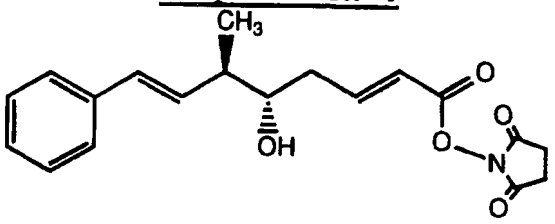
MS (FD) 457 ($M^+$,100);

$[a]_D$ +71.3° (c 10.1, $CHCl_3$);

Anal. calcd. for $C_{25}H_{35}NO_5$ requires: C,65.61;H,7.71;N,3.06%.

Found: C,65.51;H,7.56; N, 3.02%.

**Preparation 4**



To a stirred solution of silyl ether (2.50g,5.47mmol) in $CH_3CN$ (130mL) was added 48% aqueous HF (15mL) at 0°C. The solution was stirred at 0°C for 0.75h and then at room temperature for 4h. The reaction was diluted with diethylether (300mL) and washed with $H_2O$ until the wash was ~pH7. Organics were dried ($MgSO_4$) and concentrated in vacuo to give a yellow residue which was recrystallized from Et2O to give alcohol as white crystals (1.46g,78%).

$^1H$ NMR ($CDCl_3$) d 7.41-7.20 (m,$PhH_5$,3-H), 6.48 (d,J=16Hz,8-H), 6.15-6.07 (m,7-H,2-H), 3.71-3.65 (m,5-H), 2.83 (brs,$CH_2CH_2$), 2.60-2.33 (m,6-H,4-$CH_2$),1.95 (brs, 5-OH), 1.14 (d,J=6.8Hz,6-Me) ppm;

IR u (KBr)

3457,1804,1773,1735,1724,1209,1099,1067,1049,975,744,694 $cm^{-1}$;

UV (EtOH) $l_{max}$ 250 (e =20535) nm;

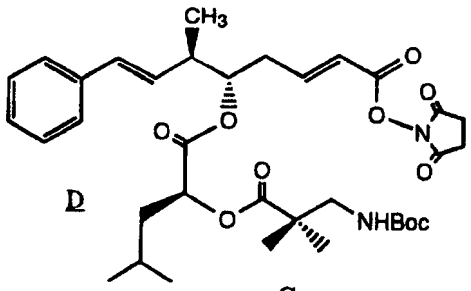
MS (FD) 343.2 ($M^+$,100);

$[a]_D$ -57.8° (c 10.56, $CHCl_3$);

Anal. calcd. for $C_{19}H_{21}NO_5S$ requires: C,66.46;H,6.16;N,4.08%. Found: C,66.49; H,6.16; N, 4.07%.

**Example 1**

A




D C

To a suspension of carboxylic acid (1.28g, 3.87mmol), in dry dichloromethane (6mL) was added EDC (742mg,3.87mmol) and DMAP (73mg,0.60mmol) and the mixture stirred at room temperature for 0.5h. A solution of alcohol (1.02g, 2.97mmol) in dichlormethane (5.5mL) was added to the reaction mixture and stirred for a further 0.3h.The reaction was diluted with $CH_2Cl_2$ (200mL) and washed with 1N aq. HCl (2x 50mL), sat. aq. $NaHCO_3$ (2x 50mL), $H_20$ (50mL). The organics were dried ($MgSO_4$) and concentrated in vacuo to leave an oily residue, which was purified by column chromatography (gradient: 10-30% EtOAc/Hexanes) to give the desired ester as a yellow solid (1.68g,79%).

$^1H$ NMR ($CDCl_3$) unit A d 7.35-7.20 (m,$PhH_5$,3-H), 6.43 (d,J=15.8Hz,8-H), 6.12 (d,J=15.9Hz,2H), 5.99 (dd,J=8.5 and15.8 Hz,7-H), 5.06-5.08 (m,5-H), 2.85 (brs,$CH_2CH_2$), 2.68-2.61 (m,6-H,4-$CH_2$), 1.13 (d,J=6.8Hz,6-Me); unit C d 5.31 (brt,NH),3.28-3.25 (m,3-$CH_2$),1.43 (s,$CMe_3$), 1.21 (s,2-Me), 1.19 (s,2-Me); unit D d 4.95 (dd,J=9.8 and 3.8Hz,2-H), 1.73-1.64 (m,3-H,4-H), 1.59-1.49 (m,3-H'), 0.85 (d,J=6.4Hz,5-Me), 0.82 (d,J=6.4,4-Me) ppm;

IR u (KBr) 3400, 2975,1743,1367,1206,1126,1145,1068 $cm^{-1}$;

MS (FD) 657 ($M^+$,100);

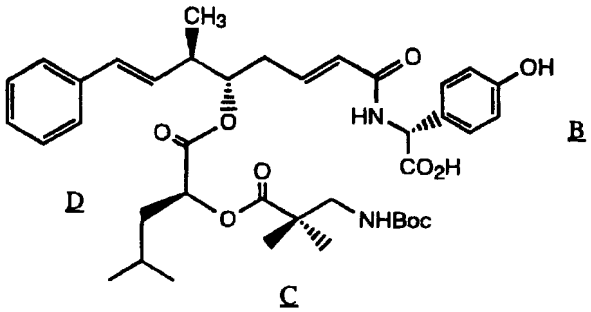
$[a]_D$+39.5° (c 10.38, $CHCl_3$);

Anal. calcd. for $C_{35}H_{48}N_2O_{10}$ requires:

C,64.01;H,7.37;N,4.27%. Found: C,64.19;H,7.27; N,4.52 %.

Example 2

A




C

To a stirred solution of active ester (150mg, 0.229mmol) in dry DMF (2.5mL) was added N,O-Bis-(trimethylsilyl)acetamide (282uL,1.143mmol) followed by D-Hydroxy-phenylglycine (57mg,0.343mmmol). The mixture was heated in a sealed tube under $N_2$ at 55 C for 20h. Reaction solution was diluted with EtOAc (180mL) and washed with 1N aq. HCl (50mL),$H_2O$ (50mL), brine (50mL), dried (MgSO4) and concentrated in vacuo to give a yellow solid. Purification of the crude solid by column chromatography (gradient: 5-20% MeOH/$CH_2Cl_2$) provided amide (122mg,75%).

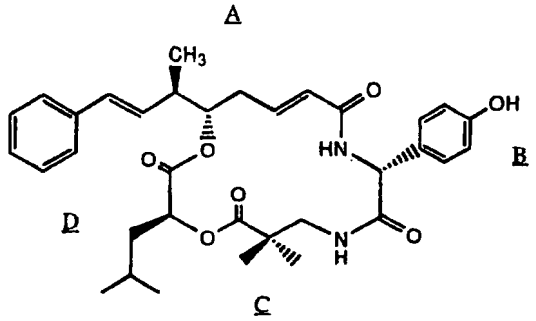
$^1H$ NMR ($CD_3OD/CDCl_3$) Unit A d 7.27-7.20 (m,$PhH_5$), 6.75-6.69 (m,3-H), 6.43 (d,J=15.9Hz,8-H), 5.96 (d,J=15.7Hz,7-H), 5.93 (d,J=15.6Hz,2-H), 4.95-4.93 (m,5-H), 2.56-2.49 (m,6-H,4-$CH_2$), 1.04 (d,J=6.8Hz,6-Me); Unit B d 7.16 (d,J=8.3Hz,$ArH_2$), 6.66 (d,J=8.2Hz,$ArH_2$), 5.62 (brt,NH)5.19-5.18 (m,2-H); Unit C d 3.15 (d,J=6.3Hz,3-$CH_2$), 1.36 (s,$CMe_3$), 1.11 (s,2-Me), 1.08 (s,2-Me); Unit D d 4.85 (dd,J=9.6 and 3.3Hz,2-H), 1.64-1.57 (m,3-H,4-H), 1.55-1.47 (m,3-H'), 0.76 (d, J=6.3Hz,5-Me), 0.73 (d,J=6.3Hz,4-Me) ppm ;

IR u (KBr) 3400,2972,1728,1672,1614,1515,1450,1416,1171,1147 $cm^{-1}$;

MS (FAB) 610.6 ($[MH_2-Boc]^+$,100);

$[a]_D$ -19.9° (c 6.53, MeOH).

Example 3




Boc amine as prepared by Example 2 (109mg,0.154mmol) was dissolved in trfluoracetic acid (5mL,5mM) and stirred at room temperature for 2h.The reaction was concentrated in vacuo and dried under high vacuum to give the trifluoroacetate salt of amine as a light brown foam. Crude amine salt (max. 0.154mmol) was dissolved in dry DMF (31mL) and diisopropylethylamine (80uL,0.462mmol), followed by pentafluorophenyl diphenyl -phosphinate (77mg,0.2mmol) added. The resulting solution was stirred at room temperature under dry $N_2$ for 15h, concentrated in vacuo and the residue purified by column chromatography (gradient: 1-4% MeOH/$CH_2Cl_2$) to provide cryptophycin as a tan solid (54mg,59%).

$^1H$ NMR ($CDCl_3$) Unit A d 7.36-7.15 (m,$PhH_5$), 6.79-6.69 (m,3-H), 6.54 (d,J=15.8,8-H), 5.98 (dd,J= 15.8 and 8.8 Hz,7-H), 5.06-5.0 (m,5-H), 2.61-2.49 (m,6-H,4-H), 2.39-2.30 (m,3-H'), 1.10 (d,J=6.8Hz,6-Me); Unit B d 7.90 (dd,J=10 and 1.68Hz,OH), 7.65 (d,J=6.3Hz,NH), 7.10 (d,J=8.5,$ArH_2$), 6.71 (d,J=8.4,$ArH_2$), 5.28 (d,J=6.5Hz,2-H), ; Unit C d 3.55-3.47 (dd,J=13.3 and 10.1Hz,3-$CH_2$), 3.00 (d,J=13.4Hz,NH) 1.19 (s,2-Me), 1.16 (s,2-Me); Unit D d 4.90 (dd,J=10 and 3.5Hz,2-H), 1.66-1.54 (m,3-H,4-H), 1.32-1.25 (m,3-H'), 0.67 (apparent t,J=7.1Hz,5-Me,4-Me) ppm;

IR u (KBr)

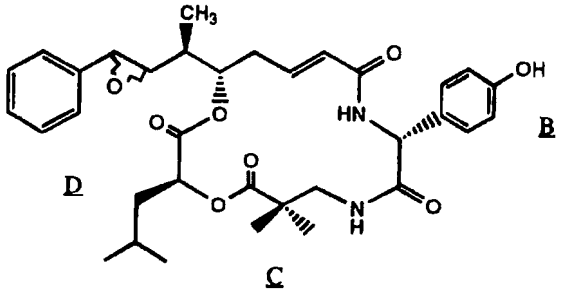
3418,3340,2960,1740,1713,16711514,1271,1198,1155,972 $cm^{-1}$;

MS (FD) 590 ($M^+$,100);

$[a]_D$+15.35° (c 3.91, $CHCl_3$).

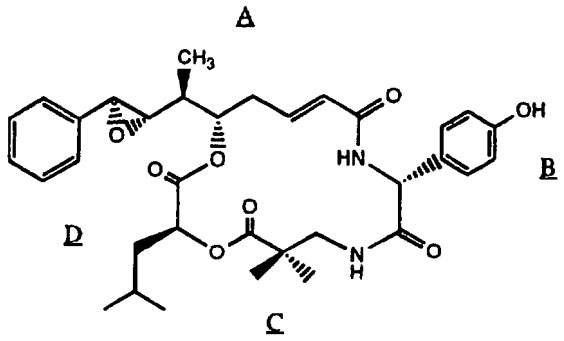
**Example 4**

A




Styrene prepared as described by Example 3 (42mg, 0.0712mmol) was suspended in dry dichloromethane (2.2mL, 0.035mM) and mCPBA (49mg, 0.285mmol) added in one portion at room temperature. Dry tetrahydrofuran (0.3mL) was added to produce a homogeneous solution. The reaction was stirred under $N_2$ at room temperture for 21h and then diluted with further $CH_2Cl_2$ (15mL). Organics were washed with 10% aq. $Na_2S_2O_5$ (10mL), sat. aq. $NaHCO_3$ (10mL), $H_2O$ (10mL), dried ($MgSO_4$) and concentrated in vacuo to give a yellow solid. Crude product was initially purified by column chromatography (gradient: 1-5% MeOH/ $CH_2Cl_2$) to give a 1: 1.15 mixture of a:b C7-C8 epoxides as a white solid (23mg, 54%).Reverse phase HPLC (column: 4.6x250mm Kromsil C18; Eluent: 60% $CH_3CN$/ $H_2O$; Flow: 1.0mL/min; UV: 220nm) separation of the a:b mixture provided a-epoxide (2.3mg, t=13.7min) and b-epoxide (5.8mg, t=12.1min) as white solids.

Example 5

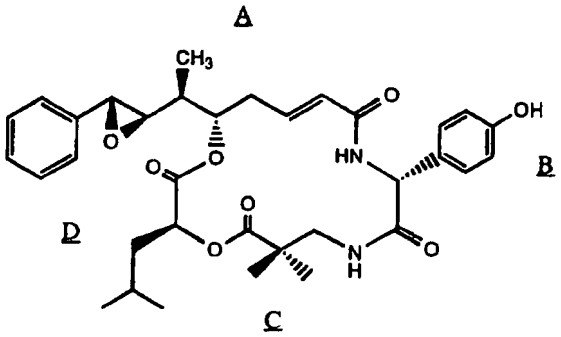



The above illustrated compound was prepared substantially as described above using the procedures of Examples 1-4

a-Epoxide:

$^1$H NMR ($CDCl_3$)

Example 6



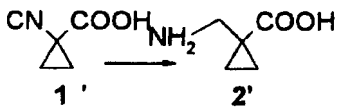

The above illustrated compound was prepared substantially as described above using the procedures of Examples 1-4

b-Epoxide:

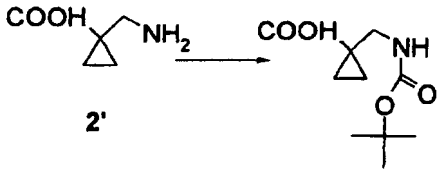
$^1$H NMR ($CDCl_3$) Unit A d 7.36-716 (m,$PhH_5$), 6.70-6.79 (m,3-H), 5.91 (dd,J=15.5 and 5.18Hz,2-H) 5.23-5.18 (m,5-H), 3.75 (d,J=1.67Hz,8-H), 2.96 (dd,J=7.4 and 2.0Hz,7-H), 2.72-2.67 (m,4-H), 2.44-2.39 (m,4-H'), 1.81-1.88 (m,6-H), 1.13 (d,J=6.9,6-Me); Unit B d 7.66 (s,NH), 7.13 (d,J=8.5Hz,$ArH_2$), 6.74 (d,J=8.5Hz,$ArH_2$), 5.27 (s,2-H); Unit C d 7.66 (s,NH),

3.49 (dd,J=13.6 and 10Hz,3-$CH_2$), 1.20 (s,2-Me),1.18 (s,2-Me); Unit D d 4.93 (dd,J=10 and 3.2Hz,2-H), 1.69-1.59 (m,3-H,4-H), 1.30-1.22 (m,3-H'), 0.79 (d,J=6.2Hz,5-Me), 0.78 (d,J=6.3Hz,4-Me) ppm.

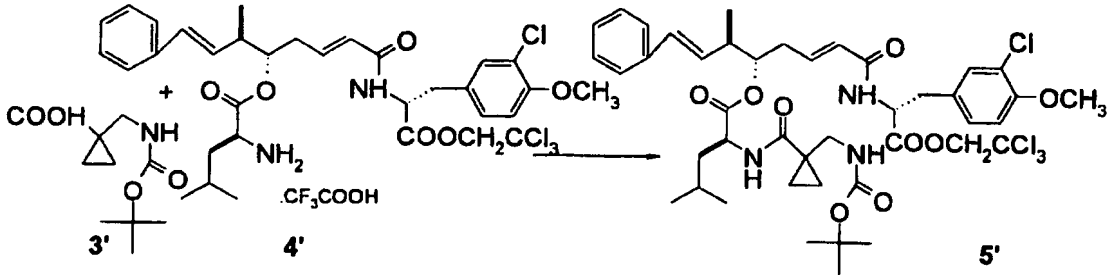
## Example 7



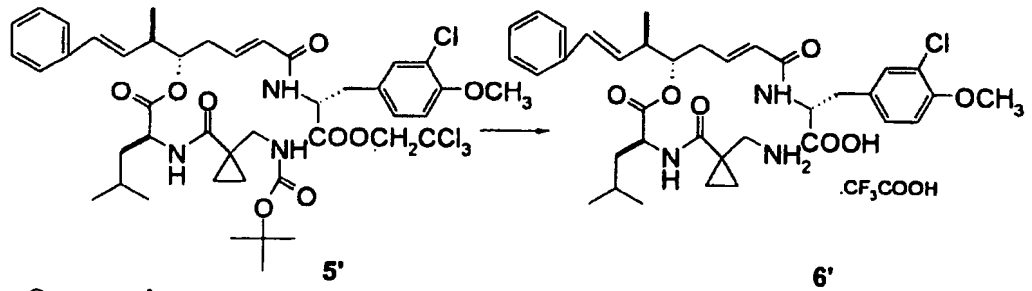

1' 2'



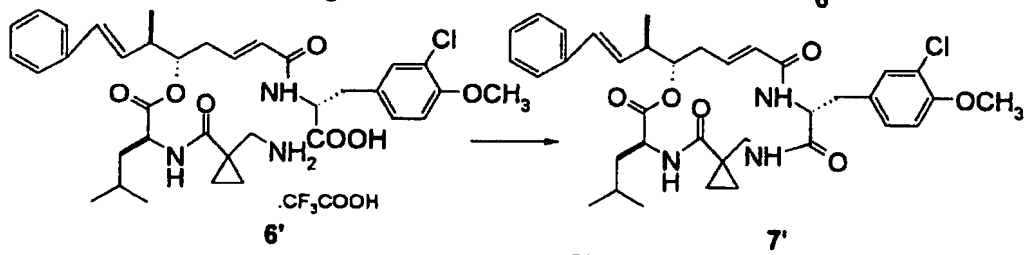

2' 3'



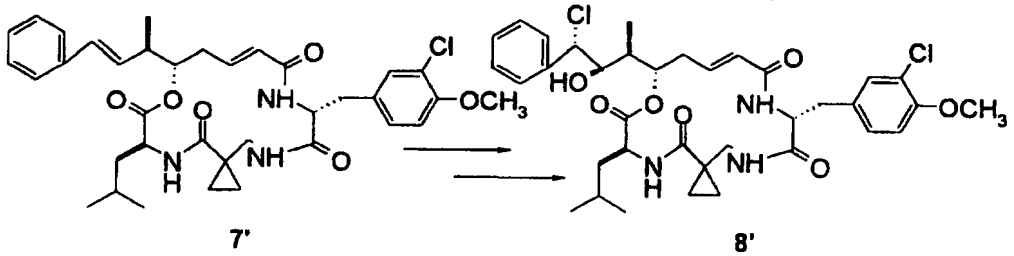

3' 4' 5'



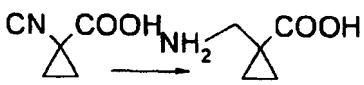

5' 6'



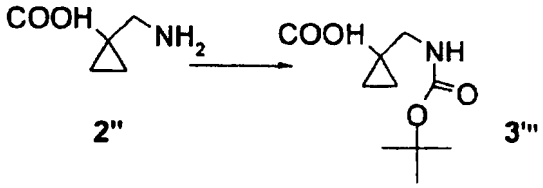

6' 7'



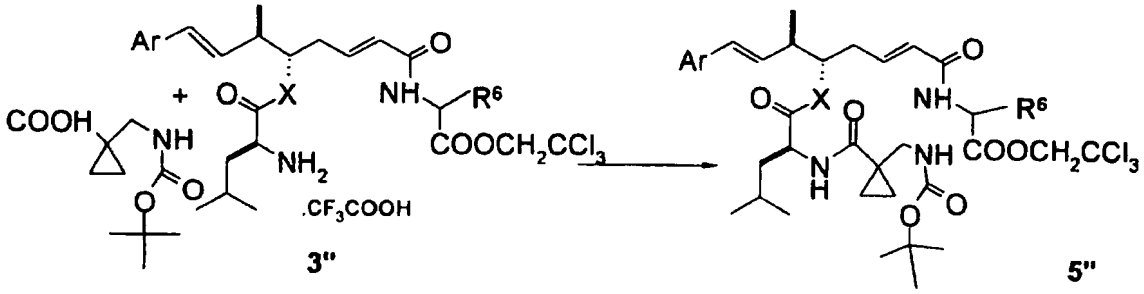

7' 8'



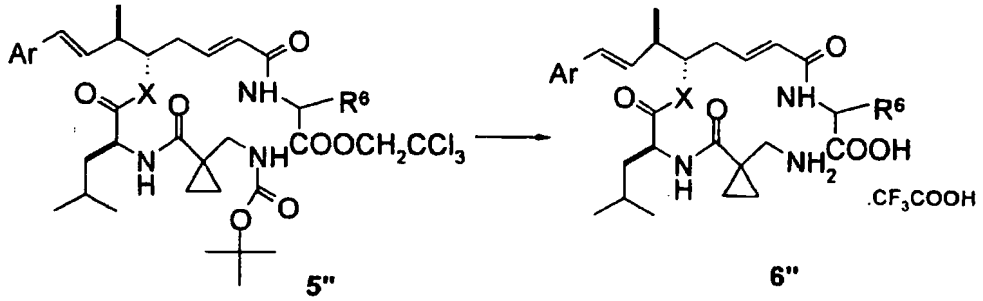
1" 2"



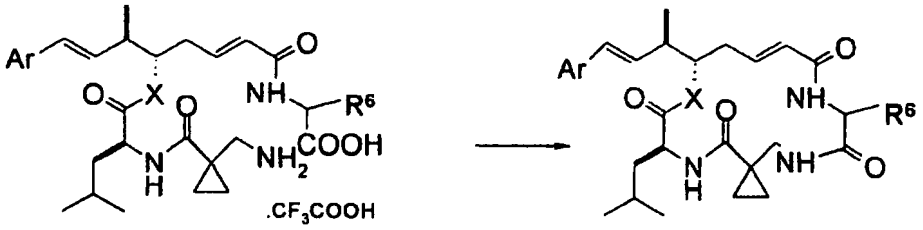
2" 3'"



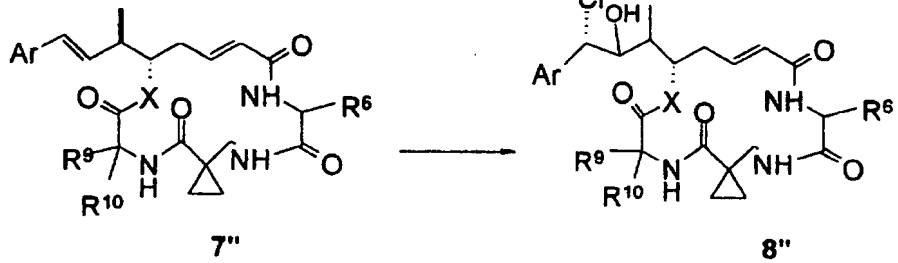
3" 5"



5" 6"



6" 7"



7" 8"

To a 500 ml Parr hydrogenator bottle were charged 3.0 g (27 mmol) of 1-cyano-1-cyclopropanecarboxylic acid **1'** (Aldrich) and 1.0 g of platinum (IV) oxide in 250 mL of glacial acetic acid. The mixture was hydrogenated at 60 psi hydrogen for 4 h. After filtering away the catalyst, the volatiles were removed *in vacuo* and the solid was dried in a vacuum oven at 75° C. This solid was then triturated in $CHCl_3$, filtered and dried to give 2.7 g (86%) of **2'** (LY257141) as a white solid.

m.p.= 261-262° C (foam, dec)

Mass (FD) M+1 = 116

To a 250 mL 24/40 round bottom flask were charged 1.5 g (13.0 mmol) of **2'** (LY257141) dissolved in 28 mL of 1,4-dioxane, 15 mL water, and 15 mL of 2N NaOH. The solution was then cooled down in an ice bath, followed by the slow addition of 3.3 mL (14.3 mmol) of di-*t*-butyl dicarbonate. The reaction was stirred at RT for 21 h. The 1,4-dioxane was removed *in vacuo* and the aqueous was diluted with additional water and layered with EtOAc. The pH of the stirring solution was adjusted to 3 using 0.5 N $NaHSO_4$. The organic layer was separated away, and the aqueous was extracted with EtOAc. The organic layers were combined, washed with brine, dried, over $Na_2SO_4$, filtered and removed *in vacuo* to give 2.6 g (93%) of **3'** (LY382186) as a white solid.

m.p.= 104-106° C

MASS (FD) M+1 = 216

After flame drying a 100 mL 14/20 3-neck round bottom flask under a nitrogen atmosphere, were charged 0.81 g (3.8 mmol) of **3'** dissolved in 10 mL of anhydrous THF, followed by the addition of 0.80 g (4.2 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and 0.64 g (4.75 mmol) of 1-hydroxybenzotriazole. Next, 10 mL of anhydrous DMF were added and a solution resulted. To this solution was then added 1.35 g (1.65 mmol) of **4'** (LY384785) and 0.31 mL (2.85 mmol) of 4-methylmorpholine dissolved in 5 mL of anhydrous DMF. The reaction was stirred at RT for 2 h. The volatiles were removed *in vacuo*, and the residue was dissolved in EtOAc and washed with 0.1 N HCl, brine, dried over $Na_2SO_4$, and removed *in vacuo*. This crude solid was purified on silica gel using flash chromatography, eluting with 20% EtOAc/Hex to give 1.14 g (77%) of **5'** (LY396076) as a white solid.

m.p.= 73-75° C

Mass (FD) M+1 = 900

To a 250 mL round bottom flask were charged 1.1 g (1.22 mmol) of **5'** (LY396076) and 4.0 g of zinc dust. The mixture was sonicated for 45 min and then stirred at RT for an additional 45 min. The reaction was filtered through celite, washed with fresh HOAc and $MeCl_2$, and the filtrate was removed *in vacuo* and pumped dry. This white solid was then dissolved in 40 mL of trifluoroacetic acid and stirred at RT for 2 h. The TFA was removed *in vacuo*, and this crude residue was purified on silica gel using flash chromatography, eluting with 20% MeOH/$CHCl_3$ to give 0.77 g (81%) of **6'** (LY396077) as a white solid.

m.p.= 131-134° C

Mass (FD) M+ = 668

To a flame dried 250 mL 14/20 round bottom flask under a nitrogen atmosphere were charged 0.76 g (0.97 mmol) of **6'** (LY396077) and 1.02 mL (5.83 mmol) of anhydrous *N,N*-diisopropylethylamine in 125 mL of anhydrous DMF. Then 0.48 g (1.26 mmol) of pentafluorophenyl diphenylphosphinate was dissolved in 18 mL of anhydrous DMF and added dropwise to the solution and the reaction was stirred at RT for 4 h. The DMF was removed *in vacuo*, and the residue was dissolved in $CHCl_3$ and washed with water, brine, dried over $NaSO_4$, and removed *in vacuo*. The crude residue was purified on silica gel using flash chromatography, eluting with 100% EtOAc to give 0.52 g (82%) of **7'** (LY396078) as a white solid.

m.p.= 114-117° C

Mass (FD) M+ = 650

After flame drying a 15 mL 14/20 round bottom flask under a nitrogen atmosphere, 0.49 g (0.75 mmol) of **7'** (LY396078) was dissolved in 5 mL of anhydrous $MeCl_2$. Next, 0.14 g (0.79 mmol) of purified 3-chloroperbenzoic acid was added and the reaction was stirred at RT for 23 h. The reaction was diluted with some additional $MeCl_2$, and washed with 10% $Na_2S_2O_5$, brine, 5% $NaHCO_3$, brine, dried over $NaSO_4$, and removed *in vacuo* to give 0.45 g (90%) of a crude white solid as a mixture of the α and β epoxides. This solid was then reacted directly without further purification. To a 50 ml 14/20 round bottom flask was dissolved 0.43 g (0.675 mmol) of the isolated epoxide mixture in 13 mL of anhydrous $CHCl_3$.

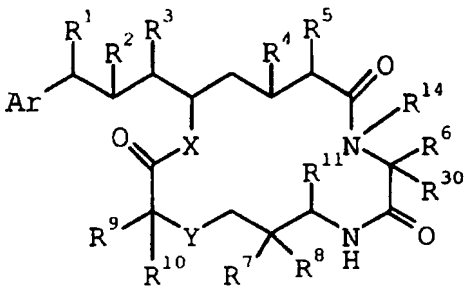
The solution was cooled down in an ice bath, followed by the addition of 0.34 mL (2.7 mmol) of chlorotrimethylsilane. The ice bath was then removed, and the reaction was stirred at RT for 2.5 h. The volatiles were removed *in vacuo*, and the crude residue was purified on silica gel using flash chromatography, eluting with 1% MeOH/EtOAc to give 0.16 g (34%) of the β-chlorohydrin **8'** as a white solid.

m.p.= 159-162° C

Mass (FD) M+ = 702

## Claims

1. The presently claimed invention provides novel compounds of Formula I



I

wherein

Ar is phenyl or any simple unsubstituted or substituted aromatic or heteroaromatic group, $C_1$-$C_{12}$ alkyl, $C_1$-$C_{12}$ alkyne;

$R^1$ is halogen, OH, $OR^{31}$, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylethio, dialkylsulfonium, sulfate, or phosphate;

$R^2$ is OH, $NH_2$, $NR^{31}$, SH; or

$R^1$ and $R^2$ may be taken together to form an epoxide ring, an aziridine ring, an episulfide ring, a sulfate ring, a cyclopropyl ring, or monoalkylphosphate ring; or

$R^1$ and $R^2$ may be taken together to form a second bond between $C_{18}$ and $C_{19}$;

$R^{31}$ is $C_1$-$C_6$ alkyl and hydrogen;

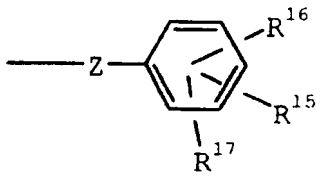
$R^3$ is a lower alkyl group;

$R^4$ is H;

$R^5$ is H;

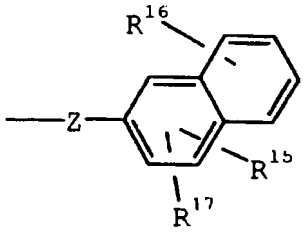
$R^4$ and $R^5$ may be taken together to form a second bond between $C_{13}$ and $C_{14}$;

$R^6$ is a substituent selected from the group consisting of B-ring heteroaromatic, substituted heteroaromatic, B-ring $(C_1$-$C_6)$alkyl, $(C_3$-$C_8)$cycloalkyl, substituted $C_3$-$C_8$ cycloalkyl, substituted $(C_1$-$C_6)$alkyl, a group of the formula III':



III'

and a group of the formula III'':



III'';

$R^7$ is selected from the group consisting of $NR^{51}R^{52}$, $R^{53}NR^{51}R^{52}$, $OR^{53}$, H and a lower alkyl group; $R^{51}$ and $R^{52}$ are independently selected from the group consisting of $C_1$-$C_3$ alkyl; $R^{53}$ is $C_1$-$C_3$ alkyl;

$R^8$ is H or a lower alkyl group;

$R^7$ and $R^8$ can optionally form a cyclopropyl ring;

$R^9$ is selected from the group consisting of H, a lower alkyl group, unsaturated lower alkyl, and lower alkyl-$C_3$-$C_5$ cycloalkyl;

$R^{10}$ is H or a lower alkyl group;

$R^9$ and $R^{10}$ together optionally form a cyclopropyl ring;

$R^{11}$ is selected from the group consisting of H, OH, simple alkyl, phenyl, substituted phenyl, benzyl, and substituted benzyl;

$R^{14}$ is H or a lower alkyl group;

$R^{15}$, $R^{16}$ and $R^{17}$ are each independently selected from the group consisting of hydrogen, ($C_1$-$C_6$)alkyl, $OR^{18}$, halo, $NR^{18'}R^{19'}$, $NO_2$, $OPO_3H_2$, $OR^{19}$phenyl, $SCH_2$phenyl, $CONH_2$, $CO_2H$, $PO_3H_2$, and $SO_2R^{23}$, and ZZ;

$R^{18}$ is selected from the group consisting of hydrogen, aryl, and $C_1$-$C_6$ alkyl;

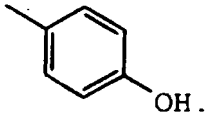
$R^{18'}$ is selected from the group consisting of hydrogen and ($C_1$-$C_6$)alkyl;

$R^{19}$ is $C_1$-$C_6$ alkyl;
$R^{19'}$ is selected from the group consisting of hydrogen and $(C_1$-$C_6)$alkyl
$R^{23}$ is selected from the group consisting of hydrogen and $(C_1$-$C_3)$alkyl;
$R^{29}$ is $(C_1$-$C_5)$alkyl;
$R^{30}$ is hydrogen or $C_1$-$C_6$ alkyl;
n is 0, 1, or 2;
p is 0, 1, or 2;
m is 0, 1, or 2;
X is selected from the group consisting of O, NH and alkylamino;
Y is selected from the group consisting of O, NH, and alkylamino;
Z is selected from the group consisting of $-(CH_2)_n-$, $-(CH_2)_p-O-(CH_2)_m-$ and $(C_3$-$C_5)$ cycloalkyl;
ZZ is selected from the group consisting of an aromatic group and a substituted aromatic group; or
a pharmaceutically acceptable salt or solvate thereof;
provided that when $R^6$ is a group of Formula III' and n is 1, then at least one of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ must be a non-hydrogen group and if only one of $R^{15}$, $R^{16}$ and $R^{17}$ is OH or $OR^{29}$ and one of the group consisting of $R^{15}$, $r^{16}$ and $R^{17}$ is halo then the remaining member of the group consisting of $R^{15}$, $R^{16}$, and $R^{17}$ must not be hydrogen or halo; or when $R^6$ is a group of Formula III' and n is 1, $R^{14}$ is a lower alkyl group.

2. A compound of **Claim 1** wherein Y is O.

3. A compound of **Claim 2** wherein X is O.

4. A compound of **Claim 3** wherein $R^6$ is a group of the formula:



5. A compound of **Claim 4** wherein $R^9$ is isobutyl and $R^{10}$ is hydrogen.

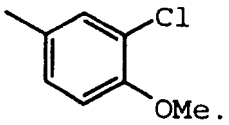
6. A compound of **Claim 5** wherein $R^8$ and $R^7$ are each independently selected from the group consisting of methyl and hydrogen.

7. A compound of **Claim 6** wherein $R^1$ and $R^2$ form an epoxide group.

8. A compound of **Claim 1** wherein none of $R^{15}$, $R^{16}$, and $R^{17}$ are $C_1$-$C_3$ alkyl.

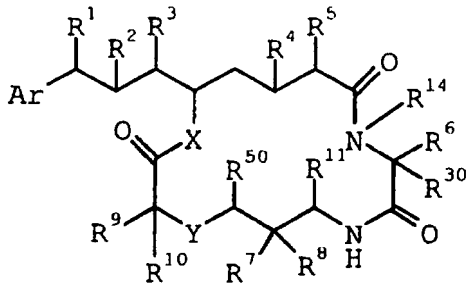
9. A compound of **Claim 8** wherein X is O.

10. A compound of **Claim 9** wherein $R^6$ is a group of the formula:



11. A compound of **Claim 10** wherein $R^8$ and $R^7$ are each methyl.

12. A compound of the Formula I'



I'

wherein

Ar is phenyl or any simple unsubstituted or substituted aromatic or heteroaromatic group, $C_1$-$C_{12}$ alkyl, $C_1$-$C_{12}$ alkyne;

$R^1$ is halogen, OH, $OR^{31}$, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, or phosphate;

$R^2$ is OH, $NH_2$, $NR^{31}$, SH; or

$R^{31}$ is $C_1$-$C_6$ alkyl and hydrogen;

$R^1$ and $R^2$ may be taken together to form an epoxide ring, an aziridine ring, an episulfide ring, a sulfate ring, a cyclopropyl ring, or monoalkylphosphate ring; or

$R^1$ and $R^2$ may be taken together to form a second bond between $C_{18}$ and $C_{19}$;

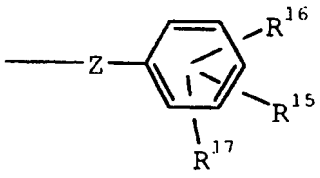
$R^3$ is a lower alkyl group;

$R^4$ is H;

$R^5$ is H;

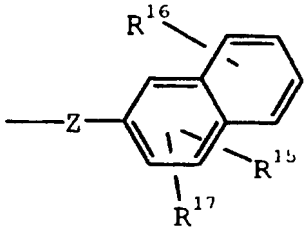
$R^4$ and $R^5$ may be taken together to form a second bond between $C_{13}$ and $C_{14}$;

$R^6$ is a substituent selected from the group consisting of B-ring heteroaromatic, substituted heteroaromatic, B-ring ($C_1$-$C_6$) alkyl, ($C_3$-$C_8$)cycloalkyl, substituted $C_3$-$C_8$ cycloalkyl, substituted ($C_1$-$C_6$) alkyl, a group of the formula **III'**:



III'

and a group of the formula III'':



III'';

$R^7$ is selected from the group consisting of H and a lower alkyl group;

$R^8$ is H or a lower alkyl group;

$R^7$ and $R^8$ can optionally form a cyclopropyl ring;

$R^9$ is selected from the group consisting of H, a lower alkyl group, unsaturated lower alkyl, and lower alkyl-$C_3$-$C_5$ cycloalkyl;

$R^{10}$ is H or a lower alkyl group;

$R^{50}$ is hydrogen or  ;

$R^{11}$ is selected from the group consisting of H, OH, simple alkyl, phenyl, substituted phenyl, benzyl, and substituted benzyl;

$R^{14}$ is H or a lower alkyl group;

$R^{15}$, $R^{16}$, and $R^{17}$ are each independently selected from the group consisting of hydrogen, $(C_1-C_6)$alkyl, $OR^{18}$, halo, $NR^{18'}R^{19'}$, $NO_2$, $OPO_3H_2$, $OR^{19}$phenyl, $SCH_2$phenyl, $CONH_2$, $CO_2H$, $PO_3H_2$, and $SO_2R^{23}$, and ZZ;

$R^{18}$ is selected from the group consisting of hydrogen, aryl, and $C_1-C_6$ alkyl;

$R^{18'}$ is selected from the group consisting of hydrogen and $(C_1-C_6)$alkyl;

$R^{19}$ is $C_1$-$C_6$ alkyl;

$R^{19'}$ is selected from the group consisting of hydrogen and ($C_1$-$C_6$)alkyl;

$R^{23}$ is selected from the group consisting of hydrogen and ($C_1$-$C_3$) alkyl;

$R^{29}$ is ($C_1$-$C_5$)alkyl;

$R^{30}$ is hydrogen or $C_1$-$C_6$ alkyl;

R is hydrogen or a group of the formula  ;

n is 0, 1, or 2;

p is 0, 1, or 2;

m is 0, 1, or 2;

X is selected from the group consisting of O, NH and alkylamino;

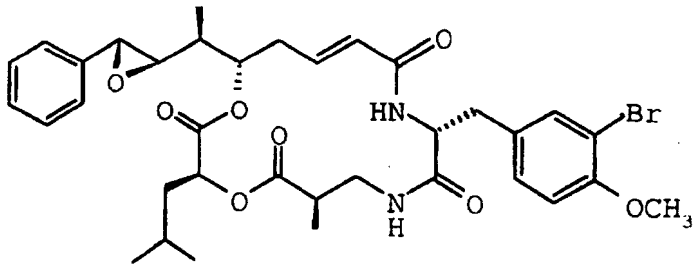
Y is selected from the group consisting of O, NH, and alkylamino;

Z is selected from the group consisting of $-(CH_2)_n-$, $-(CH_2)_p-O-(CH_2)_m-$ and ($C_3$-$C_5$)cycloalkyl;

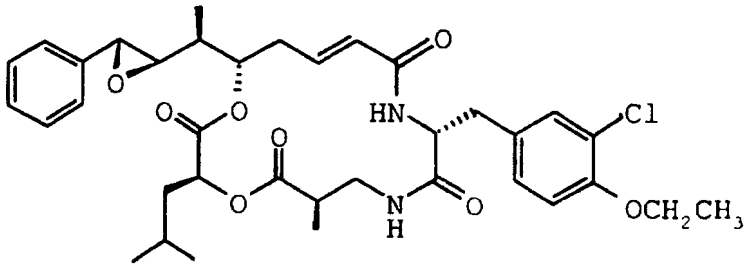
ZZ is selected from the group consisting of an aromatic group and a substituted aromatic group; or a pharmaceutically acceptable salt or solvate thereof;

provided that when $R^6$ is a group of Formula III' and n is 1, then at least one of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ must be a non-hydrogen group and if only one of $R^{15}$, $R^{16}$ and $R^{17}$ is OH or $OR^{29}$ and one of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ is halo then the remaining member of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ must not be hydrogen or halo; or when $R^6$ is a group of Formula III' and n is 1 then $R^{14}$ is lower alkyl;

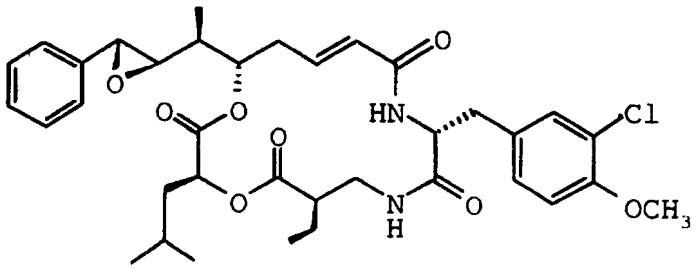
further provided that the compound is not a crytophycin selected from the group consisting of cryptophycins:



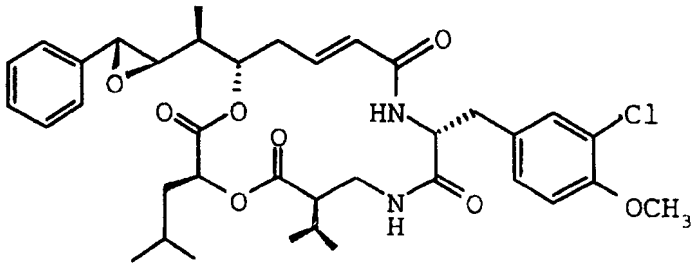
B-2,



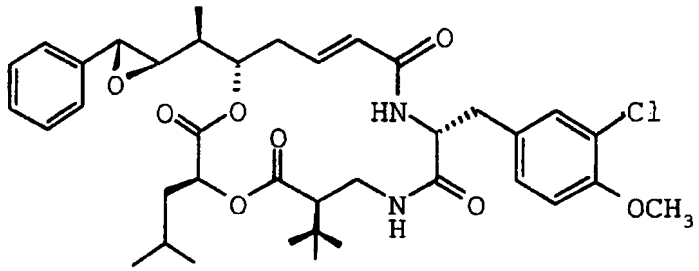
B-7,



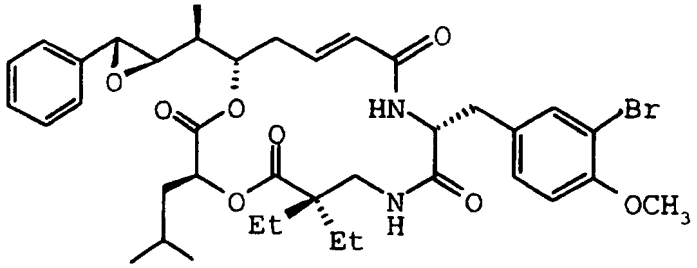
C-1,



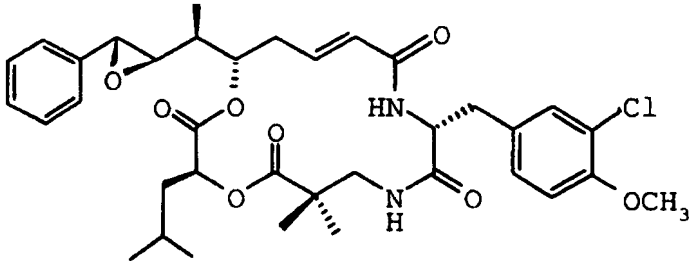
C-2,



C-3,



C-6



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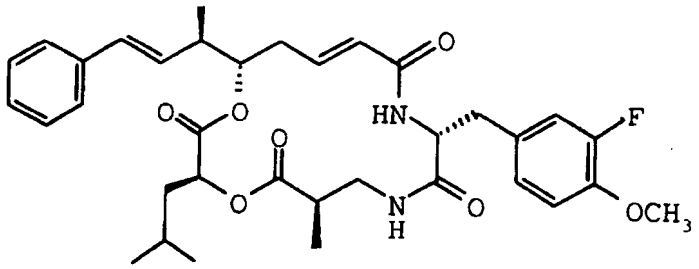

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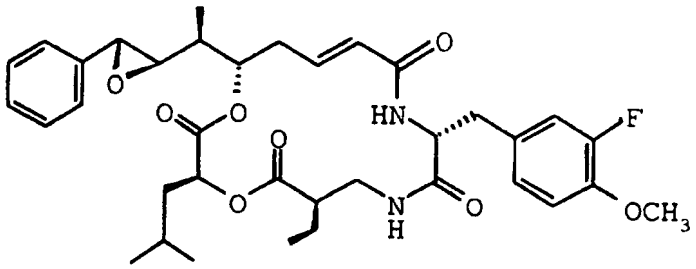

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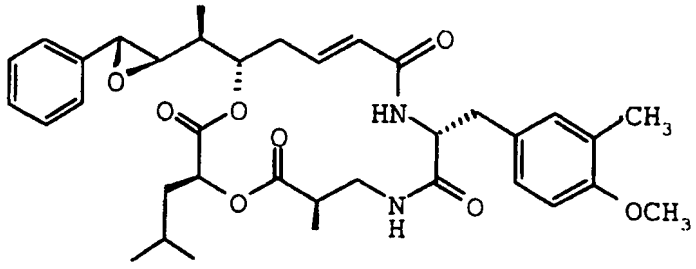

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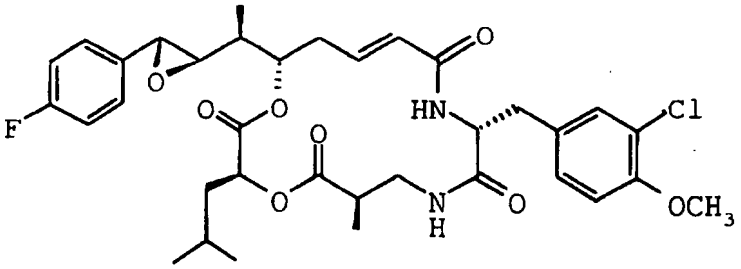

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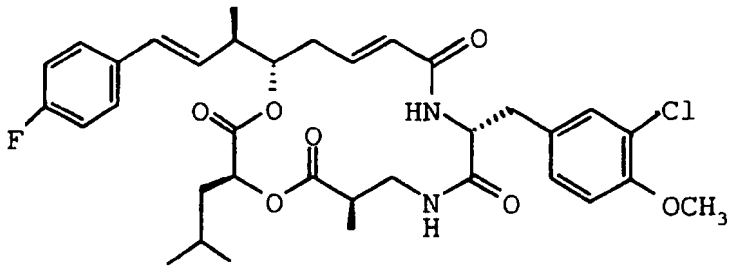

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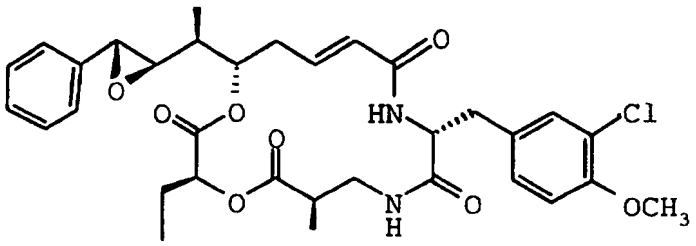

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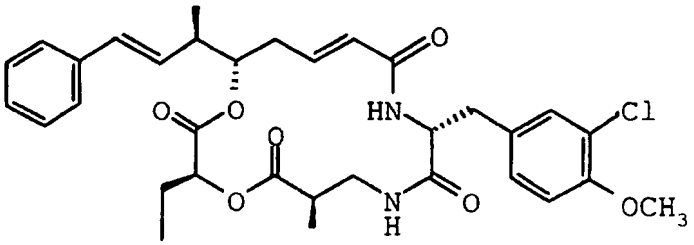

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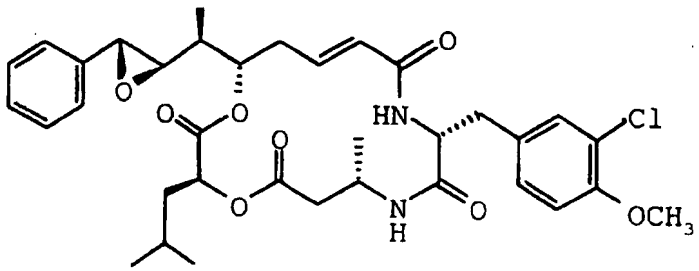

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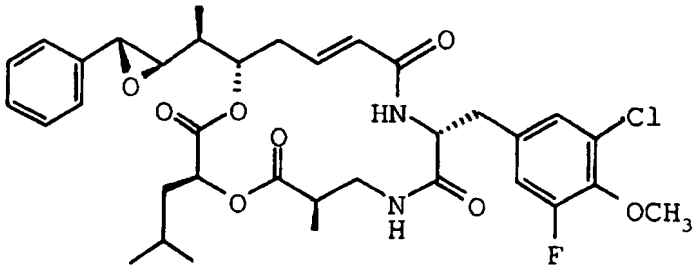

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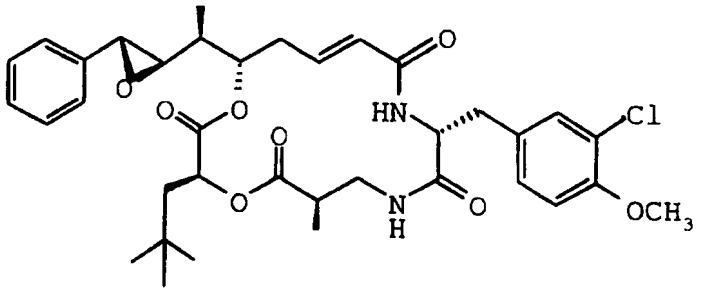
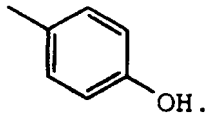


D-2

13. A compound of **Claim 12** wherein Y is O.

14. A compound of **Claim 12** wherein X is O.

15. A compound of **Claim 14** wherein $R^6$ is a group of the formula:



16. A compound of **Claim 15** wherein $R^9$ is isobutyl and $R^{10}$ is hydrogen.

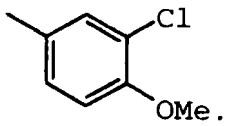
17. A compound of **Claim 16** wherein $R^8$ and $R^7$ are each independently selected from the group consisting of methyl and hydrogen.

18. A compound of **Claim 17** wherein $R^1$ and $R^2$ form an epoxide group.

19. A compound of **Claim 12** wherein none of $R^{15}$, $R^{16}$, and $R^{17}$ are $C_1$-$C_3$ alkyl.

20. A compound of **Claim 19** wherein X is O.

21. A compound of **Claim 20** wherein $R^6$ is a group of the formula:



22. A compound of **Claim 21** wherein $R^8$ and $R^7$ are each methyl.

23. A compound of **Claim 22** wherein $R^9$ is isobutyl and $R^{10}$ is hydrogen.

24. A compound of **Claim 23** wherein $R^1$ and $R^2$ form an epoxide group.

25. A compound of **Claim 12** wherein n is 0.

26. A compound of **Claim 12** wherein none of the group consisting of $R^{15}$, $R^{16}$ and $R^{17}$ is halo or $OCH_3$.

27. A compound of **Claim 26** wherein n is 0.

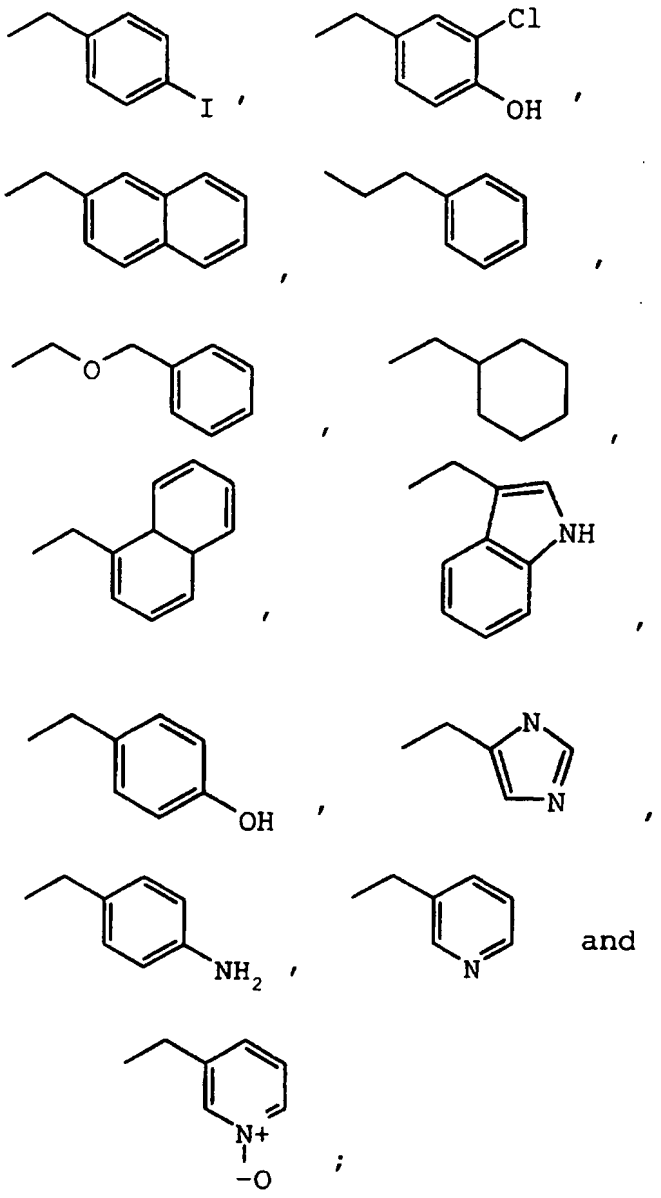
28. A compound of **Claim 26** wherein n is 2.

29. A compound of **Claim 26** wherein n is 1.

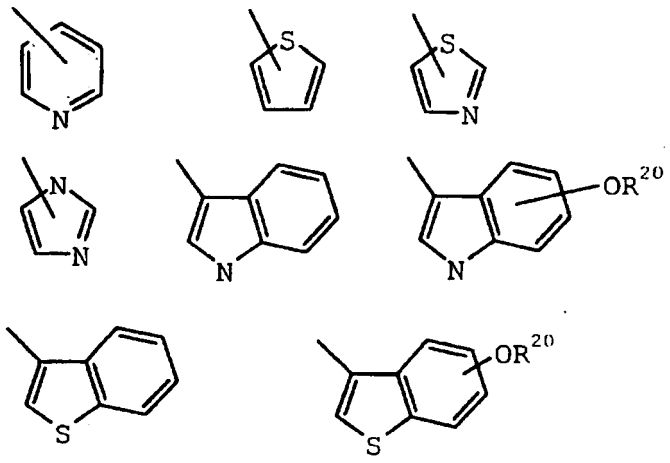
30. A compound of **Claim 14** wherein $R^{30}$ is methyl.

31. A compound of **Claim 29** wherein $R^{30}$ is hydrogen.

32. A compound of **Claim 13** wherein $R^6$ is selected from:




33. A compound of **Claim 12** wherein $R^6$ is selected from the group consisting of the following eight heteroaromatics:



34. A method for disrupting microtubule binding in a mammal comprising administering an effective amount of a compound of **Claim 1**.

35. A method for disrupting microtubule binding in vitro comprising administering an effective amount of a compound of **Claim 1**.

36. A method for treating a neoplasm in a mammal comprising administering an effective amount of a compound of **Claim 1** to a patient in need thereof.

37. A formulation comprising a compound of **Claim 1** and one or more pharmaceutically acceptable diluents or carriers therefor.

38. A method for treating a mammal suffering from or susceptible to a fungal infection, comprising administering an effective amount of a compound of **Claim 1**.

39. A method for disrupting microtubule binding in a mammal comprising administering an effective amount of a compound of **Claim 11**.

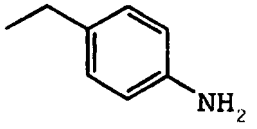
40. A method for disrupting microtubule binding in vitro comprising administering an effective amount of a compound of **Claim 11**.

41. A method for treating a neoplasm in a mammal comprising administering an effective amount of a compound of **Claim 11** to a patient in need thereof.

42. A formulation comprising a compound of **Claim 11** and one or more pharmaceutically acceptable diluents or carriers therefor.

43. A method for treating a mammal suffering from or susceptible to a fungal infection, comprising administering an effective amount of a compound of **Claim 11**.

44. A compound of **Claim 11** wherein $R^6$ is selected from the group consisting of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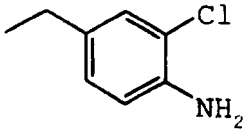
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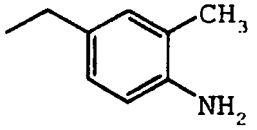
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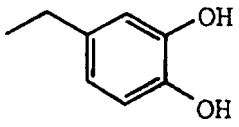
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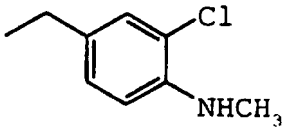
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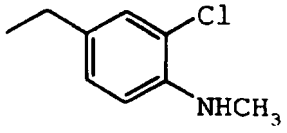
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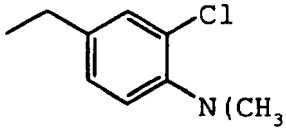
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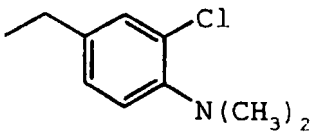
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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15245

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 31/395; C07D 273/08
US CL :514/183; 540/460, 454

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/183; 540/460, 454, 460

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CHEMICAL ABSTRACTS 1,4-dioxa-8,11-diazacyclo-hexa decane (VOLS. 112-123)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 95/17093 A1 (UNIVERSITY OF HAWAII) 29 June 1995 (29.06.95), see entire document. | 1-44 |
| X | GOLAKOTI et al. Structure Determination, Conformational Analysis, Chemical Stability Studies, and Antitumor Evaluation of the Cryptophycins. Isolation of 18 New Analogs from Nostoc sp. Strain GSV 224. J. Am. Chem. Soc. 1995, Vol. 117, pages 12030-12049, see entire document. | 1-44 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>04 NOVEMBER 1997 | Date of mailing of the international search report<br>19 NOV 1997 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>ROBERT T. BOND<br>Telephone No. (703) 308-1235 |